OTTAWA, CANADA; JUNE 22-23 JUIN 1981

WORKSHOP ON THE COMBINED EFFECTS OF XENOBIOTICS

ATELIER SUR LES EFFETS COMBINES DES XENOBIOTIQUES

NRCC / CNRC No. 18978

National Research Council Canada
Conseil national de recherches Canada

Environmental Secretariat
Secrétariat de l'environnement

Associate Committee on Scientific Criteria for Environmental Quality
Comité associé sur les critères scientifiques concernant l'état de l'environnement

Ottawa Canada
K1A 0R6

ANNOUNCEMENT
OF
PUBLICATION

WORKSHOP ON THE COMBINED EFFECTS OF XENOBIOTICS

The combined effects of toxic agents on man in his home and his workplace are of principal interest to us today. While most toxicological studies deal solely with a single pollutant or a narrow range of pollutants, the actual problem is the multiplicity of pollutants simultaneously encountered. In recognition of the need for environmental toxicologists to consolidate their thinking, papers for a workshop on this problem were solicited by the National Research Council's Associate Committee on Scientific Criteria for Environmental Quality from selected individuals and Canadian research institutions. The intent was to examine how we can give direction to a field which is essentially without productive focus.

An expert Panel of senior Canadian toxicologists and environmental scientists was convened to moderate the discussions in the workshop and to prepare suggestions for more productive directions in the Canadian research effort. The proceedings of this workshop, along with the Panel's recommendations, have recently been released as the monograph entitled "Workshop on the Combined Effects of Xenobiotics". The reports deal with the *Ah* gene complex, mixed function oxidases, and various aspects of the combined effects of polychlorinated biphenyls and asbestos, among other topics.

The Panel's report reflects on the reasons for the apparent lack of focus during the workshop and more generally in the studies on combined effects of toxic agents. The recommendations of the Panel stem from their conviction that an increase in productivity could not be achieved without a fundamental rethinking of the philosophies behind the funding of toxicological research in this country.

Reports are published in the language in which they were presented; abstracts are given in both official languages.

Publication No. NRCC 18978 (253 pages)
Available through prepaid purchase orders from

Publications, NRCC/CNRC
Ottawa, Ontario, Canada K1A 0R6

Price: $5.00

Canada

National Research Council Canada
Conseil national de recherches Canada

Environmental Secretariat
Secrétariat de l'environnement

Associate Committee on Scientific Criteria for Environmental Quality
Comité associé sur les critères scientifiques concernant l'état de l'environnement

Ottawa, Canada
K1A 0R6

NOUVELLE PUBLICATION

ATELIER SUR LES EFFETS COMBINES DES XENOBIOTIQUES

Les effets combinés des agents toxiques sur l'homme et son lieu de travail s'avèrent actuellement d'un intérêt capital. Alors que la plupart des études toxicologiques ne traitent que d'un seul polluant ou d'un nombre restreint de polluants, le problème de base est la multiplicité des polluants agissant simultanément. Pour répondre au besoin ressenti par ceux qui étudient la toxicologie de l'environnement, le Comité associé du CNRC sur les critères scientifiques concernant l'état de l'environnement a invité certains spécialistes et des institutions de recherches à examiner les modalités pour établir des priorités dans un domaine qui en a vraiment besoin.

Un groupe d'experts en toxicologie et en sciences de l'environnement a été invité à présider les débats au sein d'un atelier et à proposer des directions plus fructueuses pour les recherches canadiennes. Les comptes rendus de cet atelier, ainsi que les recommandations du groupe d'experts, viennent d'être publiés sous le titre "Atelier sur les effets combinés des xénobiotiques". Les rapports traitent du complexe génique *Ah*, des oxydases à fonctions multiples, et de divers aspects des effets combinés des polychlorobiphényles et de l'amiante, parmi d'autres sujets.

Les commentaires du groupe d'experts traitent des raisons pour le manque de priorités qui s'est révélé non seulement lors de l'atelier mais aussi en général dans les études sur les effets combinés des agents toxiques. Les recommandations du groupe d'experts découlent de leur conviction qu'on ne saurait augmenter la productivité sans réévaluer foncièrement les philosophies régissant la subvention des recherches toxicologiques au Canada.

Les rapports sont publiés dans la langue dans laquelle ils ont été communiqués; un résumé de chaque rapport paraît et en français et en anglais.

Publication nº CNRC 18978 (253 pages)
En vente, moyennant commandes prépayées,
au Service des

Publications, CNRC
Ottawa, Ontario, Canada K1A 0R6

Prix: $5.00

Canada

WORKSHOP ON THE COMBINED EFFECTS OF XENOBIOTICS

ATELIER SUR LES EFFETS COMBINES DES XENOBIOTIQUES

Ottawa, Canada
June 22-23, 1981

Ottawa, Canada
22-23 juin 1981

Edited by/Edité par
H.F. Stich
H.W. Leung
J.R. Roberts

Sponsored by:

National Research Council
of Canada

Sous l'égide du:

Conseil national de
recherches du Canada

Publication No. NRCC/CNRC 18978

of/du

Environmental Secretariat/Secrétariat de l'environnement

Crown Copyright Reserved/Droits de la Couronne réservés

1982

Available through prepaid purchase orders from/
En vente, moyennant commandes prépayées, au Service des

Publications, NRCC/CNRC
Ottawa, Ontario K1A OR6

Price/Prix: $8.00

The Associate Committee on Scientific Criteria for Environmental Quality was established by the National Research Council of Canada in response to a mandate provided by the Federal Government to develop scientific guidelines for defining the quality of the environment. The concern of the NRCC Associate Committee is strictly with scientific criteria. Pollution standards and objectives are the responsibility of the regulatory authorities and are set for the purpose of pollution control. These may be based on scientific criteria as a starting point but they also take into account the optimal socioeconomic impact of proposed measures as well as the state of existing technology.

The Associate Committee's program includes the evaluation of available information on the probability of effects of contaminants on receptors together with the related fundamental principles and scientific knowledge. In this work particular attention is directed to receptors and contaminants (and their interactions) important to Canada. This Canadian approach is necessary because evaluations made in other countries or regions will not always be applicable to the particular circumstances prevailing in Canada.

Members of the Associate Committee, its Subcommittees and Expert Panels, serve voluntarily and are selected for their individual competence and relevant experience with due consideration for a balance among all sectors in Canada. Responsibility for the quality of study documents rests with the Associate Committee. Each report is carefully reviewed according to a multistage procedure established and monitored by the National Research Council of Canada in order to preserve objectivity in presentation of the scientific knowledge. Publication and distribution of the report are undertaken only after completion of this review process.

Comments on Associate Committee documents are welcome and will be carefully reviewed by the Expert Panels. It is foreseen that these scientific criteria may be revised from time to time, as new knowledge becomes available.

All documents published by the Associate Committee are published in both French and English.

Le Comité associé sur les critères scientifiques concernant l'état de l'environnement a été créé par le Conseil national de recherches du Canada en réponse à un mandat du gouvernement fédéral visant à élaborer des directives scientifiques pour définir la qualité de l'environnement. Ce Comité s'intéresse strictement aux critères scientifiques, les normes de pollution et les objectifs étant la responsabilité des autorités de réglementation et étant établis dans le but de lutter contre la pollution. Ces normes et objectifs sont d'abord basés sur des critères scientifiques, mais ils tiennent compte également de l'impact socio-économique optimal des mesures proposées ainsi que de l'état de la technologie existante.

Le programme du Comité associé comprend l'évaluation des données disponibles relatives à la probabilité des effets de produits de contamination sur les organismes récepteurs, ainsi que l'appréciation des principes fondamentaux et des connaissances scientifiques qui y sont apparentés. On s'intéresse surtout aux produits de contamination et aux organismes récepteurs (et leurs interactions) qui sont importants pour notre pays, car les évaluations faites dans d'autres pays ou dans d'autres régions ne s'avèrent pas toujours pertinentes pour le Canada.

Les membres du Comité associé, de ses Sous-comités et de ses Groupes d'experts siègent à titre volontaire et sont choisis suivant leur compétence individuelle et leur expérience applicable, tout en tenant compte de l'équilibre qu'il faut conserver entre tous les secteurs au Canada. La responsabilité de la qualité des documents d'étude appartient au Comité associé. Chaque rapport est soigneusement revu suivant une méthode comprenant des étapes multiples, méthode qui a été établie et qui est contrôlée par le Conseil national de recherches du Canada, afin de conserver l'objectivité dans la présentation de connaissances scientifiques, la publication et la diffusion d'un rapport n'étant effectuées qu'après révision complète.

Nous invitons les intéressés à émettre des commentaires sur les documents du Comité associé, commentaires qui seront ensuite soigneusement revus par des Groupes d'experts. On envisage la révision périodique de ces critères scientifiques au fur et à mesure des nouvelles connaissances.

Tous les ouvrages publiés par le Comité associé le sont en français et en anglais.

TERMS OF REFERENCE OF THE SUBCOMMITTEE ON PESTICIDES AND INDUSTRIAL ORGANIC CHEMICALS

(1) The preparation of reports critically evaluating and collating the available cause-effect relationships which permit the estimation of the environmental risks associated with the use of synthetic organic chemicals in Canada - these documents will provide criteria for the evaluation of both present and future use patterns, and

(2) the identification of gaps in available criteria and preparation of recommendations for future research in these areas.

To meet this mandate, the Subcommittee has identified a number of priority compounds for consideration. Comprehensive reports are prepared to bring into perspective the diverse and somewhat obscure observations found in the literature. The documents consist of reviews, summarizing the available criteria and the research required. They also contain as supporting material the available data on which the overview is based.

The mandate of the Subcommittee does not include the setting of objectives and standards, the development of risk assessment, or the establishment of registered uses for pesticides in Canada. Therefore, it does not permit consideration of the engineering, economic, or legal aspects. The Subcommittee is only concerned with scientific information and environmental criteria. The documents do provide one of the components necessary in the consideration and development of risk assessments. Other agencies, both federal and provincial, have the actual responsibility for making decisions about the uses of specific synthetic organic chemicals in Canada and hence they have the task of bringing about an acceptable balance between the risks and benefits associated with the use of such chemicals by our society.

Inquiries concerning the activities of the Subcommittee should be directed to the Environmental Secretariat of the National Research Council of Canada, Ottawa, Canada.

MANDAT DU SOUS-COMITE DES PESTICIDES ET PRODUITS CHIMIQUES ORGANIQUES INDUSTRIELS

(1) Rédaction de rapports critiques faisant l'appréciation et la collation des diverses relations de cause à effet, permettant l'estimation de produits chimiques organiques de synthèse au Canada. Ces documents établiront des critères servant à évaluer les modes d'utilisation présents et futurs; et

(2) Identification des lacunes dans les critères disponibles et préparation de recommandations pour la recherche future dans ces domaines.

Pour remplir ce mandat, le Sous-comité a dressé une liste d'un certain nombre de composés prioritaires qui feront l'objet d'études. De vastes rapports mettront en perspective les observations diverses et quelque peu obscures dont fait état la littérature. Les documents comprennent des comptes rendus qui résument les critères disponibles et la recherche nécessaire. Ils contiennent également les données sur lesquelles est basée la vue d'ensemble.

Le mandat du Sous-comité ne comprend ni l'élaboration d'objectifs et de normes, ni le développement d'évaluations des risques, ni l'établissement de modes d'emploi homologués au Canada. Le Sous-comité ne tient nullement compte des aspects techniques, économiques, esthétiques, juridiques ou politiques, mais s'intéresse uniquement à l'information scientifique et aux critères environnementaux. Les documents constituent l'un des éléments nécessaires à la considération et à l'évaluation des risques. D'autres organismes, tant fédéraux que provinciaux, ont la responsabilité de décider des usages des produits chimiques organiques de synthèse au Canada et il leur appartient de trouver un juste milieu entre les risques et les avantages associés à l'emploi de ces produits dans notre société.

Veuillez adresser toutes demandes de renseignements concernant les activités du Sous-comité au: Secrétariat de l'environnement du Conseil national de recherches du Canada, Ottawa (Canada).

PREFACE

The combined effects of toxic agents on man in his home and his workplace are of principal interest to us today. While most toxicological studies deal solely with a single pollutant or narrow range of pollutants the actual problem is the multiplicity of pollutants simultaneously encountered. In recognition of the need for environmental toxicologists to consolidate their thinking, papers have been solicited from selected individuals and research institutions which examine how we can give directions to a field which is essentially without productive focus. The papers selected for this workshop deal primarily with analyses of the biological principles required to order our thinking and relate our understanding of combined effects to the human situation.

Les effets combinés des agents toxiques sur l'homme et son lieu de travail s'avèrent actuellement d'un intérêt capital. Alors que la plupart des études toxicologiques ne traitent que d'un seul polluant ou d'un nombre restreint de polluants, le problème de base est la multiplicité des polluants agissant simultanément. Pour répondre au besoin ressenti par ceux qui étudient la toxicologie de l'environnement nous avons invité certains spécialistes et des institutions de recherches à examiner les modalités pour établir les priorités dans un domaine qui en a vraiment besoin. Leurs rapports choisis pour cet atelier traitent principalement de l'analyse des principes biologiques nécessaires pour faire avancer les recherches dans ce domaine et pour améliorer notre compréhension des effets combinés sur l'homme lui-même.

TABLE OF CONTENTS
TABLE DES MATIERES

		PAGE
I.0	PANEL'S CRITIQUE - The Need for New Directions in Toxicological Funding/COMMENTAIRES DU GROUPE D'EXPERTS - Le besoin d'une nouvelle politique en ce qui concerne les subventions dans le domaine toxicologique	11
II.0	WORKSHOP PROCEEDINGS/COMPTES RENDUS DE L'ATELIER	19
II.1	INTRODUCTORY REMARKS/DISCOURS D'ACCUEIL H.F. Stich	21
II.2	PAPERS PRESENTED/CONFERENCES	35
	The Ah Gene Complex: A Model for Studying Specific Interactions of Carcinogens and Toxic Chemicals/Le complexe génique Ah: Un modèle pour l'étude d'interactions particulières entre carcinogènes et toxiques chimiques A.B. Okey and D.W. Nebert	37
	Methodological Approaches for Interaction Studies: Potentiation of Haloalkane-Induced Hepatotoxicity/Démarches méthodologiques pour des études d'interaction: Potentialisation de l'hépatotoxicité induite par les haloalcanes G.L. Plaa and W.R. Hewitt	67
	Endogenous Mechanisms for the Regulation of Hepatic Microsomal Drug and Steroid Mixed Function Oxidases/Mécanismes endogènes assurant la régulation des oxydases à fonctions multiples des microsomes hépatiques responsables de la dégradation des medicaments et des stéroïdes G.D. Bellward, R.C.K. Pak, L.S. Gontovnick, B.L. Warren, M.J.P. Finlayson and G.I. Sunahara	97
	Polychlorinated Biphenyls: Species and Tissue Specificities of the Induction of Monooxygenases in Rats, Mice, Rabbits and Humans/Les polychlorobiphényles: Specificité de l'induction des monooxygénases rélative aux espèces et aux tissus, chez le rat, la souris, le lapin et l'homme A.P. Alvares, J.L. Eiseman, T.-H. Ueng and A. Kappas	127
	Polychlorinated Biphenyls: Effects of Structure on Biologic and Toxic Activity/Polychlorobiphényles: Effets de la structure sur l'activité biologique et toxique S. Safe, A. Parkinson, L. Robertson, L. Safe and S. Bandiera	151

TABLE OF CONTENTS (CONT'D)
TABLE DES MATIERES (SUITE)

PAGE

Mixed Function Oxidases of Fish Liver and Mammalian Lung: Interactions with Xenobiotics/Les systèmes d'oxydases à fonctions multiples dans le foie chez les poissons et dans les poumons chez les mammifères: Interactions avec les xénobiotiques 177
F.C.P. Law

The Effect of Asbestos on the Bioactivation of Polynuclear Aromatic Hydrocarbons/Effet de l'amiante sur l'activation biologique des hydrocarbures aromatiques polycycliques 207
C. Kandaswami and P.J. O'Brien

Some Emerging Principles in the Toxicological Analysis of the Combined Effects of Xenobiotics/Certains nouveaux principes pour l'analyse toxicologique des effets combinés des xénobiotiques 237
E. Farber

I.0 PANEL'S CRITIQUE - THE NEED FOR NEW DIRECTIONS IN TOXICOLOGICAL FUNDING

PANEL MEMBERS

Dr. H.F. Stich (Chairman of Panel)	British Columbia Cancer Research Centre
Dr. J. Brodeur	Université de Montréal
Dr. C.A.R. Dennis	Prairie Institute of Environmental Health
Dr. D. Grant	Health and Welfare Canada
Dr. D. McCalla	McMaster University
Dr. G.L. Plaa	Université de Montréal
Dr. J.R. Roberts (Secretary)	National Research Council of Canada

Man encounters chemicals in combinations and the effects that result from such exposures can reflect the specific nature of the chemical mix encountered. While in some instances the toxic effect may be due to only one component of the mixture, in others the combination of chemicals present defines the outcome. On the other hand, hazard is normally assessed upon the basis of tests of individual chemicals, not tests of the combinations that are likely to occur in real-life situations. Toxicologists have usually avoided studies on combinations of chemicals because of their complexity in terms of experimental design and interpretation, as well as the cost associated with such experiments. In fact, there is no prospect for the comprehensive toxicity testing of all chemicals even as individual substances, let alone as mixtures. This situation has resulted in important gaps in the knowledge of chemical interactions which make assessments and regulatory decisions particularly difficult. While regulators and concerned public representatives are continually calling for refined studies, in actuality few data bases are being developed to allow assessments to be made within the context of the total pollutant mix. Simply stated, our current understanding of the fundamental scientific principles of chemical interactions and methodology is insufficiently developed to permit the required assessments.

One of the better developed statements of the problem can be found in the U.S. National Academy of Sciences' report on the assessment of chemical hazard to workers on ships (NAS 1980). There, a single chemical is clearly not of prime concern because of the complex mixtures of oils, fumigants, fuels, paints and solvents used in the industry.

The toxicologists of the National Academy of Science's panel concluded that it was impossible to provide exposure criteria for the situations found on ships. They found that, while the general pharmacological principles governing combined effects could be catalogued, the applicability of these principles to a given pollutant mixture could not be established because of our limited understanding of the specific mode of action of most chemicals. Explicitly, they argued that they were uncertain about which basic toxicological mechanisms are applicable in a particular situation.

It was the intention of the organizers of this workshop on the Combined Effects of Xenobiotics to provide a forum in which attention could be focused on laboratory research projects which show promising approaches to this complex problem. It was not the intention to examine the complementary epidemiological approaches. While the papers emphasize the role of microsomal enzymes in the appearance of disease in man and animals, the workshop did provide a microcosm in which to assess the productivity of the current research effort and to determine positive directions for productive Canadian research. Our understanding of the role of microsomal enzymes is one of the more advanced aspects of toxicology and it is our opinion that the observations developed by the participants of this workshop about the needed research directions are just as applicable to other less developed mechanisms by which the effects of mixtures are mediated. The following observations can be made from this workshop.

1. The focus of the toxicological studies to date, with the exception of studies on oncogenicity, has usualy been on the clarification of broad mechanistic principals of general scientific interest. While functional changes at the biochemical level of general interest have received considerable attention per se, little attention has been given to the linkage between the biochemical manifestations and the ultimate end point of interest --disease in man or other animals. In fact, the linkage is often of secondary interest to many investigators although it is this linkage that is of paramount importance in assessment.

2. The preponderance of the toxicological research and the scientific thought, while of high quality, is not directed to the comprehensive elucidation of the fundamental principles by which chemicals interact but rather to highly specific and sometimes narrow aspects of how biochemical functions are affected by chemicals.

3. The discussion of the participants at the workshop identified the need to give greater focus to the fundamentals of predicting chemical interactions rather than to descriptive studies of basic biochemical mechanisms in which little

attention is given to their linkage to disease, or to descriptive studies of ad hoc combinations of chemicals. The participants stressed that it is futile to expect to resolve the dilemmas surrounding the assessment of combined effects without a basic shift in the emphasis of toxicological funding. They argue that a greater emphasis should be given to the funding of studies that attempt to integrate our present understanding of biochemical mechanism into a basis for the prediction of the outcome of chemical interactions. Additionally, they argue that funding policy should be changed to promote studies that stress socially relevant end points.

Arguments for the adoption of a funding policy emphasizing the fundamentals of chemical interactions are based upon pragmatism and economics. A two-year carcinogenicity study using rats can cost up to one million dollars for a single chemical and the costs of testing possible permutations of chemicals escalate exponentially if a purely prospective approach is taken. Ultimately, such a labor-intensive exercise is limited by manpower requirements and it is applicable only to high-priority situations where a well characterized combination of chemicals is believed to represent a potential hazard. Thus, this approach does not provide a viable basis for the ultimate resolution of the general problems associated with the presence of innumerable combinations of chemicals in the environment. On the other hand, the funding of studies solely examining the fundamentals of chemical interactions, while productive in the long term, may not provide immediate answers where major problems are perceived. There is a need for both kinds of studies, but it is our opinion that in the long term, the studies on the fundamentals provide the only approach to a general resolution of the problem.

At present, the funding of studies of a purely exploratory nature seems to be a relatively unproductive approach for resolving the issues surrounding the impact of mixtures of chemicals on man. The myriad choices of end points (i.e. diseases in man or animals) and classes of chemicals that could be studied require that any new program of funding avoids encouraging work of a simply esoteric nature. The necessary focus could be provided through the identification of classes of chemicals whose interactions are deemed a priority for Canadian society. The choice of end points must reflect a consensus on both the needs of Canadian society and the depth of our understanding of the biochemical principles associated with a disease. The pragmatics that are used to develop any consensus must also be employed in the future for the reassessment of the priorities in light of our continually expanding understanding of chemical interactions.

An urgent problem is how we are going to proceed until approaches to testing are refined. A shift in the direction of funding will serve to steer the research onto a more productive path. The opinion of the panel is that we have to proceed without delay. The task of testing chemical combinations is phenomenal and we must not allow ourselves to be restrained by old practices; rather, we must aim at

progress that can lead to the needed refinements in the testing protocols which will provide more informative data bases. Ultimately, we also have to recognize that it is beyond our resources to test all chemical combinations. In numerous situations the only way to reduce risk in the short term may be to minimize exposure.

REFERENCES

NAS. 1980. Principles of toxicological interactions associated with multiple chemical exposures. National Academy of Sciences Press, Washington, D.C.

I.0 COMMENTAIRES DU GROUPE D'EXPERTS - LE BESOIN D'UNE NOUVELLE POLITIQUE EN CE QUI CONCERNE LES SUBVENTIONS DANS LE DOMAINE TOXICOLOGIQUE

MEMBRES DU GROUPE D'EXPERTS

Dr. H.F. Stich (Président)	British Columbia Cancer Research Centre
Dr. J. Brodeur	Université de Montréal
Dr. C.A.R. Dennis	Prairie Institute of Environmental Health
Dr. D. Grant	Santé et Bien-être social Canada
Dr. D. McCalla	Université McMaster
Dr. G.L. Plaa	Université de Montréal
Dr. J.R. Roberts (Secrétaire)	Conseil national de recherches du Canada

L'être humain se trouve exposé à des mélanges de produits chimiques et les effets résultant de ces expositions peuvent témoigner de la nature particulière du mélange chimique en question. Même si dans certains cas l'effet toxique peut provenir d'un seul composant du mélange, dans d'autres cas c'est le mélange des produits présents qui détermine la toxicité. D'autre part, on évalue généralement les dangers en étudiant des produits chimiques individuels et non les mélanges de ces produits susceptibles de se produire dans des situations réelles. Les toxicologues ont habituellement négligé d'étudier les mélanges de produits en raison de leur complexité en termes de techniques expérimentales et d'interprétation, et en raison des coûts qu'entraînent de telles expériences. En fait, il n'existe aucune étude exhaustive de prévue sur la toxicité de tous les produits chimiques, même pris individuellement, et encore moins sur les mélanges de ces produits. Par conséquent, notre connaissance des interactions chimiques est largement insuffisante, ce qui complique les estimations et les décisions à prendre sur la règlementation des produits chimiques. Les personnes chargées d'élaborer des règlements et les agents gouvernementaux s'intéressant au problème exigent continuellement des études plus poussées; cependant, en réalité on n'accumule guère de données permettant d'effectuer des estimations dans le contexte du mélange total de polluants. Ainsi, notre connaissance actuelle des principes scientifiques fondamentaux des interactions chimiques et de la méthodologie est insuffisante pour nous permettre d'effectuer les évaluations nécessaires.

On peut trouver l'un des comptes rendus les mieux élaborés sur le sujet dans un rapport de la National Academy of Sciences des E.-U. traitant de l'évaluation du danger constitué par les produits chimiques pour les personnes travaillant sur les bateaux (NAS 1980). Dans ce cas là, un seul produit chimique n'est pas la préoccupation essentielle en raison des mélanges complexes d'huiles, de produits de fumigation, de combustibles, de peintures et de solvants utilisés dans l'industrie. Selon les conclusions des toxicologues du groupe d'experts de la National Academy of Sciences, il était impossible de fournir des critères d'exposition critique pour les situations prévalant sur les bateaux. Ils ont constaté que, même si l'on pouvait répertorier les principes pharmacologiques généraux régissant les effets combinés, on ne pouvait appliquer ces principes à un mélange donné de polluants en raison de notre compréhension limitée du mode d'action de la plupart des produits chimiques. En termes plus clairs, ils ont affirmé leur incertitude quant à l'applicabilité des mécanismes toxicologiques fondamentaux à des situations particulières.

Les organisateurs de cet atelier sur les effets combinés des xénobiotiques avaient l'intention de prévoir un débat consacré aux projets de recherche en laboratoire pouvant présenter des solutions d'avenir à ce problème épineux. Il n'était pas question d'étudier les solutions épidémiologiques complémentaires. Tandis que les communications ont mis l'accent sur le rôle des enzymes microsomales dans l'apparition de la maladie chez l'homme et chez l'animal, l'atelier a présenté un microcosme permettant d'évaluer l'efficacité des efforts de recherche actuels et de déterminer des orientations positives en vue de recherches fructueuses au Canada. Notre compréhension du rôle des enzymes microsomales constitue l'un des aspects les plus avancés de la toxicologie; nous estimons que les observations émises par les participants de cet atelier sur la nécessité de trouver de nouvelles orientations pour la recherche peuvent tout autant s'appliquer à d'autres mécanismes moins élaborés intervenant dans la toxicité de ces mélanges chimiques. Cet atelier a permis de tirer les observations suivantes:

1. Jusqu'à présent, les études toxicologiques, à l'exception des études sur le caractère oncogène, se sont généralement orientées vers les mécanismes d'action. Même si les modifications fonctionnelles et les mécanismes biochimiques ont été largement étudiés, on s'est peu intéressé au lien entre les manifestations biochimiques et le point final d'intérêt, soit les affections dont sont atteints les hommes et les animaux. En fait, ce lien est souvent d'un intérêt secondaire pour de nombreux chercheurs bien que pour une évaluation il s'avère de prime importance.

2. La plupart des recherches toxicologiques et la pensée scientifique, quoique de haute qualité, ne se sont pas principalement orientées à élucider, de façon complète, les principes essentiels selon lesquels les produits chimiques interagissent, mais se sont intéressés à des aspects très

spécifiques et parfois étroits du fonctionnement biochimique modifiés par des interactions chimiques.

3. Les débats de cet atelier ont identifié le besoin de se consacrer plus à fond aux questions essentielles permettant de prévoir les interactions chimiques plutôt qu'aux études descriptives des mécanismes biochimiques à partir desquels on ne peut pratiquement pas, faute de données suffisantes, établir de lien avec la maladie, ou aux études descriptives des combinaisons ad hoc de produits chimiques. Les participants ont insisté sur le fait qu'il est inutile d'espérer résoudre les difficultés entourant l'évaluation des effets combinés sans modifier au départ l'orientation du financement des recherches toxicologiques. Il y aurait lieu de se consacrer davantage à des études visant à intégrer notre connaissance actuelle des mécanismes biochimiques à des données de base permettant de prévoir les interactions chimiques. En outre, de telles études devraient insister sur des points particulièrement importants sur le plan social.

Les discussions concernant l'adoption d'une politique de financement mettant l'accent sur des principes fondamentaux reposent sur le pragmatisme et l'économie. Une étude du pouvoir carcinogène portant sur deux ans et utilisant des rats peut coûter jusqu'à un million de dollars pour un simple produit chimique et les coûts des différentes permutations possibles de produits chimiques croissent de façon exponentielle si l'on utilise une méthode purement prospective. Enfin, une étude si ardue est limitée par les exigences de main-d'oeuvre et ne peut être appliquée qu'à des situations hautement prioritaires où l'on estime qu'un mélange bien défini de produits chimiques représente un danger possible. Ainsi, cette étude ne fournit pas de base viable permettent de résoudre finalement les problèmes d'ordre général causés par la présence d'inombrables produits chimiques dans l'environnement. D'autre part, le financement des études examinant seulement les principes fondamentaux des interactions chimiques, même s'il s'avère productif à long terme, ne peut fournir de réponse immédiate aux principaux problèmes perçus. Il y a lieu d'effectuer ces deux types d'études, mais nous estimons qu'à long terme les études des principes fondamentaux fournissent la seule méthode pour résoudre ces problèmes.

Actuellement, le financement d'études de nature purement exploratoire semble une approche relativement peu fructueuse pour résoudre les questions concernant les effets des mélanges de produits chimiques sur l'homme. Le multiple choix des conséquences finales (c.-à.-d. les maladies de l'homme et des animaux) et les classes de produits chimiques qui pourraient être étudiées exigent que tout nouveau programme de financement évite d'encourager des recherches de nature simplement ésotérique. On pourrait se concentrer à l'identification des classes de produits chimiques dont les interactions sont, estime-t-on,

une priorité pour la société canadienne. Le choix des conséquences finales doit refléter un consensus sur les besoins de la société canadienne et sur l'étendue de notre connaissance des principes biochimiques associé à une maladie. Le pragmatisme utilisé pour élaborer tout consensus doit également être employé à l'avenir pour réévaluer les priorités à la lumière de nos nouvelles connaissances des interactions chimiques.

La façon dont nous allons procéder jusqu'à ce que l'on améliore les méthodes d'étude constitue un problème urgent. Un changement d'orientation du financement des recherches permettrait de diriger ces dernières vers une voie plus productive. Selon le groupe d'experts, il faut agir sans tarder. L'étude des mélanges de produits chimiques représente un travail considérable et nous ne devons pas être limités par de vieilles méthodes; nous devons viser plutôt à améliorer les méthodes de recherche susceptibles de produire des données plus valables. Enfin, nous devons également reconnaître que nous n'avons pas les ressources pour étudier tous les mélanges de produits chimiques. Dans de nombreux cas, la seule façon de diminuer le risque à court terme peut être de rendre l'exposition minimale.

REFERENCES

NAS. 1980. Principles of toxicological interactions associated with multiple chemical exposures. National Academy of Sciences Press, Washington, D.C.

II.0 WORKSHOP PROCEEDINGS/COMPTES RENDUS DE L'ATELIER

II.1 INTRODUCTORY REMARKS

H.F. Stich

Human populations are exposed daily to an onslaught of chemical and physical mutagens, carcinogens and teratogens. At least 60,000 of the approximately 4 million registered compounds are in common use. It is therefore not surprising that the public is scared and the regulators are irritated by the lack of solid data to arrive at a proper regulatory decision to protect man. Despite an intensive effort to identify and remove industrial and agricultural carcinogens and mutagens, little attention has been paid to the naturally occurring carcinogens and mutagens. The curious attitude prevailed that the edible compounds of our diet, vitamins, essential trace elements and chemicals cannot have genotoxic or carcinogenic properties. However, a simple epidemiological study of gastrointestinal carcinomas points to the involvement of naturally occurring carcinogens rather than man-made ones. For example, during the Edo and early Meiji periods, when Japan was virtually closed to foreign imports, the people relied on their own food sources which were void of preservatives and colorants. In spite of their "uncontaminated" food products, the incidence of gastric carcinomas in those periods was comparable to that of today when the Japanese consume a diet loaded with synthetic additives and industrial contaminants (Sugimura 1979).

The percentage contribution of man-made agents to the total carcinogenic and mutagenic load of the environment is unknown. The available data do not even permit a reasonable guess. Despite the millions of chemicals to which man is exposed, only a few thousand have been tested for mutagenicity and of these only a few hundred have been tested for carcinogenicity. This scarcity of data is shockingly inadequate to enable us to assess the health hazard of a particular environment. The situation is still more depressing when one considers that the testing of this tiny percentage of chemicals required the effort of all existing monitoring or screening laboratories.

It is becoming evident that reasonable progress can be achieved only by setting strict priorities. There are difficulties with this proposal. What criteria will be used in setting priorities? Who will evaluate the results? Who will implement the conclusions? One other unresolved issue concerns the usefulness of *in vitro* short-term tests for predicting a genetic or carcinogenic hazard (Stich and San 1981). More than 6000 compounds have been tested with the *Salmonella typhimurium* mutagenicity test, about 4000 with various chromosome aberration assays, about 500 with several yeast systems, and approximately 400 with the DNA repair tests. Their usefulness is based mainly on a correlation between results of carcinogenicity tests on rodents and results of *in vitro* tests. According to Ames the correlation is satisfactory, whereas according to Legator, that link is less than perfect. Whatever one's attitude, there are two major unresolved issues which require immediate attention. Firstly, there is

only about a 60% agreement between carcinogenicity tests performed on rodents (Campbell 1980). Even the results between different inbred mouse strains can differ significantly. Thus one must assume that carcinogenicity results obtained on rats and mice may frequently give a wrong prediction when extrapolated to a human population. The possibility that *in vitro* short-term tests could be even better predictors for human carcinogenesis than the classical carcinogenesis test on inbred rodents should be explored. Secondly, it is presently unknown which of the many genetic lesions (e.g. frameshift mutants, substitution mutants, chromosome aberrations, mitotic recombinations) is involved in neoplastic transformation of mammalian and human cells. It is therefore necessary to search for the genotoxic endpoint which is best correlated with human carcinogenesis.

The logistics of estimating the carcinogenic and mutagenic properties of single compounds is staggering. An in depth examination of even the most important compounds already taxes all the existing testing facilities. The difficulties will be compounded when testing programs are extended to analyze chemical mixtures. However, this issue cannot be ignored since we are exposed to numerous complex mixtures rather than to single chemicals. The latter pattern may be found only at the workplace. It seems, therefore, of paramount importance to obtain information on the daily exposure of human populations to dietary chemicals. Only a profound understanding of the "background" exposure patterns will help estimate the contribution of man-made chemicals to the total genotoxic burden. A short glimpse into food chemistry will convince everyone of the enormous complexity of many common food items. Today's markets offer over 10,000 items which are boiled, fried, roasted and simmered in uncountable ways, all of which could enhance or reduce their genotoxic capacities. Table 1 shows a list of genotoxic compounds recently uncovered in regular food products. The complexity of the situation becomes particularly evident considering that the aroma of roasted coffee contains more than 80 derivatives of only one chemical group, namely the furans (Table 2). All of the 12 examined furan compounds were found to possess genotoxic activity. The other major genotoxic components of the coffee aroma are the pyrazines, thiophenes and thiazoles (Maga 1975a,b). Moreover, each cup of coffee contains 250-260 mg of chlorogenic acid (Challis and Bartlett 1975) which we found to have mutagenic, clastogenic and gene convertogenic activities (Stich *et al.* 1981c) and a host of other phenolic compounds. Thus a cup of coffee contains thousands of different chemicals. If we assume that about 5% of all chemicals have mutagenic properties, then one should expect to find in roasted coffee more than 100 genotoxic agents.

Considering the relatively large amount and number of genotoxically active compounds in coffee or tea including chlorogenic acid, phenols, phenolic acids, cinnamic acids (Stich *et al.* 1981a), flavonoids (Uyeta *et al.* 1981), pyrazines (Stich *et al.* 1980b) and furans (Stich *et al.* 1981b), one should have expected to find a much stronger association between these beverages and cancer than has actually been recorded (Fraumeni *et al.* 1971; Simon *et al.* 1975; Howe

Table 1. Examples of genotoxic chemicals found in food.

Pyrazine[α]	Galangin[δ]
2-Methylpyrazine	Kaempferol
2-Ethylpyrazine	Morine
2,5-Dimethylpyrazine	Fisetin
2,6-Dimethylpyrazine	Quercetin
1,5-Dimethyl-2,3,6,7-tetrahydro-1H, 5H-biscyclopentapyrazine	Rhamnetin
	Robinetin
	Myricetin
Furan[β]	Tamarixetin
Furfural	
Furfural alcohol	Lucidin[ε]
2-Furyl methyl ketone	Purpurin
5-Hydroxy methyl furfural	Ermodin
2,5-Dimethyl furan	Catenarin
5-Methyl furfural	Dichloroanthrarufin
2-Methyl furan	Dinotrochrysazin
Safrole[γ]	Thiazolidine[ζ]
Estragole	2-Methylthiazolidine
ρ-Asarone	2-Ethylthiazolidine
Anethole	2-n-Propylthiazolidine
Eugenol	2-Isopropylthiazolidine
Myristicin	2-n-Butylthiazolidine
Elemicin	2-Isobutylthiazolidine
3-Amino-1, 4-dimethyl-5H-pyrido {4, 3-b}-indole (trp-P-1)[δ]	Benzo(a)pyrene[η]
	Anthracene
3-Amino-1-methyl-5H-pyrido {4, 3-b}-indole (Trp-P-2)	Benz(a)anthracene
	Phenanthrene
2-Amino-6-methyldipyrido {1, 2-a:3', 2'-d}-imidazole (Glu-P-1)	Benzo(b)fluoranthene
	Dibenzo(a,h)pyrene
	Chrysene
2-Amino-3-methylimidazo-{4, 5-f} quinoline (IQ)	Glyoxal[θ]
	Diacetyl
2-Amino-3, 4-dimethyl-imidazo {4, 5-f} quinoline (MeIQ)	1,2-Cyclohexanedione
	Maltol
	Ethyl maltol
	Kojic acid

[α] Stich et al. (1980a).

[β] Stich et al. (1980b).

[γ] Miller et al. (1979).

[δ] Sugimura (1979).

[ε] Brown (1980).

[ζ] Mihara and Shibamoto (1980).

[η] Lo and Sandi (1978).

[θ] Bjeldanes and Chew (1979).

Table 2. Furans identified in roasted coffee (Maga 1979).

Furan	1-(2'-Methyl-2'-furyl)-3-butanone
2-Methylfuran	3-(2'-Furyl)-propenal
3-Methylfuran	1-(2'-Furyl)-propane-1,2-dione
2,5-Dimethylfuran	1-(2'-Furly)-butane-1,2-dione
2-Ethylfuran	1-(5'-Methyl-2'-furyl)-propane-1,2-dione
2-Propylfuran	1-(5'-Methyl-2'-furyl)-butane-1,2-dione
2-Butylfuran	Methylthio furoate
2-Pentylfuran	5-Methyl-2-furfurylnitrile
2-Acetylfuran	2,2',Difuryl
2-Propionylfuran	2,2'-Difurfurylmethane
5-Methylfuran	5-Methyl-1-2-furyl-2'-furfurylmethane
5-Methyl-2-acetylfuran	Bis(5-methyl-2-furyl)-methane
5-Methyl-2-propionylfuran	4-(2'-Furyl)-3-butene-2-one
2-Butylfuran	2-Furfuryloxyacetone
1-(2'-Furyl)-2-propanone	2-Furylacetone
1-(2'-Furyl)-2-butanone	2-Isobutylfuran
1-(2'-Furyl)-3-butanone	3-(2'-Furyl)propanal
Benzofuran	Tetrahydrofuran
2-Methylbenzofuran	2-Methyltetrahydrofuran
3-Phenylfuran	2-Acetyltetrahydrofuran
Furfuryl alcohol	2-Isopropenyl-5-vinyl-5-methyltetrahydrofuran
5-Methyl-2-methyl-furfurylsulfide	2,3-Dihydrobenzofuran
Furfurylmethyether	2-Methyltetrahydrofuran-3-one
Methyl furoate	2,4,5-Trimethyl-3(2H)-furanone
Ethyl furoate	2,5-Dimethyl-3(2H)-furanone
Propyl furoate	(-)-(2R),5R-trans-2-Vinyl-2-methyl-5-(1'-hydroxy-1-methylethyl)-tetrahydrofuran
Butyl furoate	(+)-(2R,58)-cis-2-Vinyl-2-methyl-5-(1'-hydroxy-1'-methylethyl-)tetrahydrofuran
Isobutyl furoate	4-(2'-Furyl)-but-3-en-2-one
Isopentyl furoate	2-(2'-Furyl)-pyrazine
2-Methylbutyl furoate	2-Furfuryl formate
Dimethyl furoate	2-Furfuryl acetate
Difurfuryl ether	N-Furfuryl-2-pyrrole
5-Methyldifurfuryl ether	2-Vinylfuran
Furfurlmercaptan	2-Vinyl-5-methylfuran
Furfurylmethylsulfide	2-Vinyl-3-methylfuran
Thioethyl furoate	2-Vinyl-4-methylfuran
Difurfurylsulfide	
Furfural	

Table 2. (cont'd).

1-(5'-Methyl-2'-furyl)-2-propanone	2-Vinyl-3,5-dimethylfuran
1-(5'-Methyl-2'-furyl)-2-butanone	2-Vinyl-4,5-dimethylfuran
	2-Propenylfuran
	2-Methyl-5-propenylfuran
2-Isobutenylfuran	
2-Methyl-5-isopropylfuran	
2,3,5-Trimethylfuran	
2-Isopropylfuran	
2-Furfuryl formate	
2-Furfuryl acetate	
2-Furfuryl propionate	
5-Methylfuran	
4-(2'-Furyl-5'-methyl)-butan-2-one	
3-(2'-Furyl-5'-methyl)-propan-2-one	
4-(2'-Furyl)-butan-2-one	
5-Hydroxymethylfurfural	
5-Methylfurfuryl alcohol	
4-(2'-Furyl)-butan-3-one	
2-Furyl-(2'-methyl-5'-furyl)methane	
2-Furfuryl-2'-methylbutyrate	

et al. 1980). Thus the question as to the predictive value of in vitro genotoxicity results for human carcinogenesis must be raised. Can these results which were obtained on single chemicals be used to calculate the hazard posed by a complex mixture?

One of the difficulties lies in the observations that the application of two or more compounds will not necessarily result in simple additive effects. An example of a synergistic action is shown in Table 3. The combined application of arecoline, an alkaloid of the carcinogenic betel nut, and the mutagenic quercetin found in the spices of a betel quid induces a clastogenic effect which is considerably greater than the sums of each individual action (Stich et al. 1981b). Such effects are usually described as "potentiating" or "enhancing", in contrast to an "additive" effect, which is the sum of each independent activity. Potentiation may not be an uncommon phenomenon. They can occur between mutagens belonging to different molecular groups and between mutagens and compounds that, by themselves, lack any genotoxic activity. For example, diethylstilbestrol, a non-mutagen, enhanced the mutagenicity of 2-acetylaminofluorene (Allaben et al. 1979), harman and norharman potentiated a series of different mutagens (Ashby and Styles 1978; Nagao et al. 1977) and transition metals, Cu^{2+}, Mn^{2+}, Fe^{3+}, have been found to enhance the clastogenic activity of several naturally occurring chemicals (Stich et al. 1978, 1979, 1980a; Whiting et al. 1980).

Most food products contain thousands of different chemicals, including many genotoxically active compounds. Can all genotoxic agents potentiate the clastogenic or mutagenic actions of others within a complex mixture? If this should prove to be the case, then the minimum dose of a compound which contributes to the overall genotoxicity of a mixture will be considerably smaller than the minimum dose which induces a detectable genotoxic effect when each chemical is tested individually. In such a situation, the soundness of setting "no-effect", "allowable" or "acceptable" doses on the basis of the results obtained on individual compounds should be seriously questioned. To avoid confusion, it may even be necessary to distinguish between "genotoxic range", which would be applicable to individually assayed chemicals, and "co-genotoxic range", which may include concentrations at which a chemical can exert a potentiating effect in mixtures (Stich et al. 1981d).

Apart from mutagens, recombinogens, clastogens and carcinogens, a complex mixture will contain antimutagens, anti-carcinogens, tumor promotors and DNA repair inhibitors and a host of compounds which can alter the activity of enzymes necessary to produce the ultimate electrophiles that can interact with target molecules. Examples of antimutagenic activities of food components are shown in Figures 1 and 2. The test system consists of nitrosating methylurea at pH 2 and detecting the resulting mutagenic agents with the S. typhimurium assay. The inhibitory action of phenolics (Figure 1) and tannic acid (Figure 2) was measured by adding them to the nitrosation mixtures.

Table 3. Chromosome aberrations in Chinese Hamster Ovary cells following concurrent application of arecoline and quercetin.

Arecoline (µg/mL)	Chromosome Aberrations[α] Quercetin (µg/mL) -	50	25
-	0.8(0.01)[β]	1.7	0.5(0.02)
400	13.9(0.23)	72.1(1.55)	65.2(1.68)
200	3.6(0.04)	20.0(0.29)	10.6(0.27)
100	1.0(0.01)	10.7(0.15)	3.8(0.03)
50	1.0(0.01)	1.1(0.01)	0.9(0.01)

[α] The frequency of chromosome aberrations is expressed as (a) the percentage of metaphase plates with a least one chromatic break and one chromatid exchange, and (b) the average number of chromatid breaks and exchanges per cell (figures in parentheses).

[β] The figures represent the chromosome aberrations in Chinese Hamster Ovary cell cultures which were not exposed to a clastogenic agent.

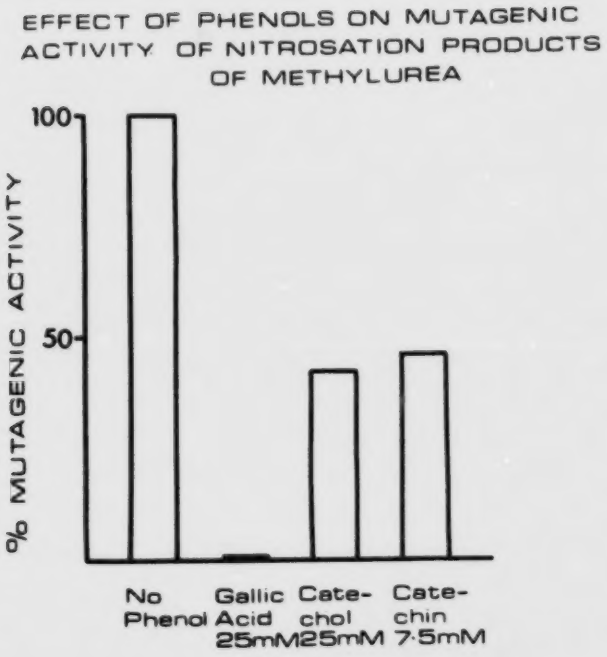


Figure 1. Effect of phenols on mutagenic activity of nitrosation products of methylurea.

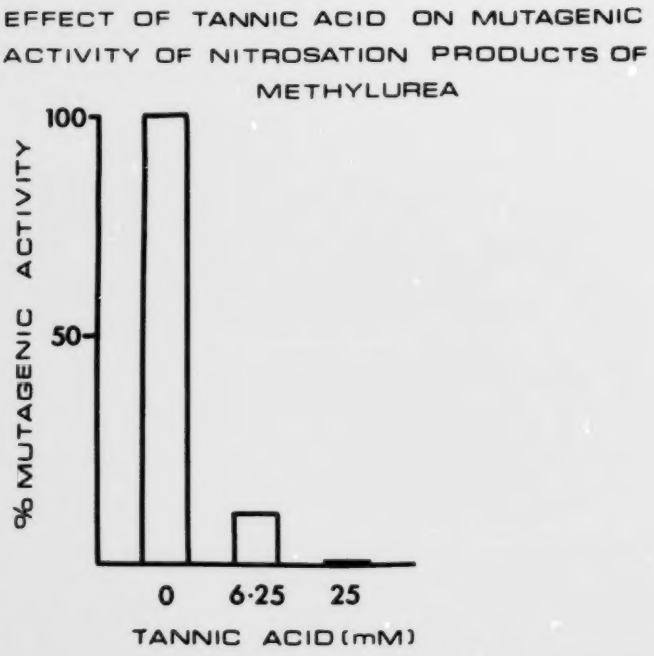


Figure 2. Effect of tannic acid on mutagenic activity of nitrosation products of methylurea.

A proper assessment of the overall genotoxicity of a complex mixture is fraught with apparently insurmountable difficulties. The genotoxicity of a complex mixture may not represent a simple summation of the mutagenic and antimutagenic activities of its components (Hermann et al. 1980; Pelroy and Peterson 1979; Shahin and Fournier 1978). In fact, its toxicity may undergo continuous modification during passage through different organs within an organism. Finally, the number of permutations is so great when several compounds start to interact that it becomes virtually impossible to design an experiment which can be handled by one laboratory.

Carcinogenicity tests on rodents are too expensive and cumbersome for examining complex mixtures. A battery of short-term in vitro test systems may be used for studying interactions of a few compounds. But for properly assessing the health hazard of complex mixtures a search for alternative approaches must be initiated.

REFERENCES

Allaben, W.T., Louie, S.C. and Lazear, E.J. 1979. Synergistic effect of diethylstilbestrol on the mutagenicity of 2-acetylaminofluorene and N-hydroxy-acetylaminofluorene in the Salmonella assay system. Cancer Lett. 7: 109-111.

Ashby, J. and Styles, J.A. 1978. Comutagenicity, competitive enzyme substrates, and in vitro carcinogenicity assays. Mutat. Res. 54: 105-112.

Bjeldanes, L.F. and Chew, H. 1979. Mutagenicity of 1,2-dicarbonyl compounds: maltol, kojic acid, diacetyl and related substances. Mutat. Res. 67: 367-371.

Brown, J.P. 1980. A review of the genetic effects of naturally occurring flavonoids, anthraquinones and related compounds. Mutat. Res. 75: 243-277.

Campbell, T.C. 1980. Chemical carcinogens and human risk assessment. Fed. Proc. 39: 2467-2484.

Challis, B.C. and Bartlett, C.D. 1975. Possible cocarcinogenic effects of coffee constituents. Nature (London) 254: 532-533.

Fraumeni, J.F., Jr., Scotto, J. and Dunham, L.J. 1971. Coffee-drinking and bladder cancer. Lancet II: 1204.

Hermann, M., Chaude, O., Waill, N., Bedouelle, H. and Hofnung, M. 1980. Adaptation of the Salmonella/mammalian microsome test to the determination of the mutagenic properties of mineral oils. Mutat. Res. 77: 327-339.

Howe, G.R., Burch, J.D., Miller, A.B., Cook, G.M., Esteve, J., Morrison, B., Gordon, P., Chambers, L.W., Fodor, G. and Winsor, G.M. 1980. Tobacco use, occupation, coffee, various nutrients and bladder cancer. J. Natl. Cancer Inst. 64: 701-713.

Lo, M.T. and Sandi, E. 1978. Polycyclic aromatic hydrocarbons (polynuclear) in foods. Residue Rev. 69: 35-86.

Maga, J.A. 1975a. The role of sulphur compounds in food flavour: Part I: thiazoles. Edited by T.E. Furia. CRC Crit. Rev. Food Sci. Nutr. pp. 153-176.

Maga, J.A. 1975b. The role of sulphur compounds in food flavour. Part II. triophenes Edited by T.E. Furia. CRC Crit. Rev. Food Sci. Nutr. pp. 241-270.

Maga, J.A. 1979. Furans in food. CRC Crit. Rev. Food Sci. Nutr. 11: 355-400.

Mihara, S. and Shibamoto, T. 1980. Mutagenicity of products obtained from cysteamine-glucose browning model systems. J. Agric. Food Chem. 28: 62-66.

Miller, J.A., Swanson, A.B. and Miller, E.C. 1979. The metabolic activation of safrole and related naturally occurring alkenylbenzenes in relation to carcinogenesis by these agents. In Naturally occurring carcinogens-mutagens and modulators of carcinogenesis. Edited by E.C. Miller, J.A. Miller, L. Hirono, T. Sugimura and S. Takayama. Univ. Park Press, Baltimore. pp. 111-125.

Nagao, M., Yahagi, T., Honda, M., Seino, Y., Matsushima, T. and Sugimura, T. 1977. Demonstration of mutagenicity of aniline and toluidine by norharman. Proc. Jpn. Acad. 53: 34-37.

Pelroy, R.A. and Peterson, M.R. 1979. Use of Ames Test in evaluation of shale oil fractions. Environ. Health Perspect. 30: 191-203.

Shahin, M.M. and Fournier, F. 1978. Suppression of mutation induction and failure to detect mutagenic activity with Athabasca tar sand fractions. Mutat. Res. 58: 29-34.

Simon, D., Yen, S. and Cole, P. 1975. Coffee drinking and cancer of the lower urinary tract. J. Natl. Cancer Inst. 54: 587-591.

Stich, H.F. and San, R.H.C. (Eds) 1981. Short-term tests for chemical carcinogens. Springer-Verlag, New York.

Stich, H.F., Wei, L. and Lam, P. 1978. The need for a mammalian test system for mutagens: action of some reducing agents. Cancer Lett. 5: 199-204.

Stich, H.F., Wei, L. and Whiting, R.F. 1979. Enhancement of the chromosome-damaging action of ascorbate by transition metals. Cancer Res. 39: 4145-4151.

Stich, H.F., Stich, W. and Acton, A.B. 1980a. Mutagenicity of fecal extracts from carnivorous and herbivorous animals. Mutat. Res. 78: 105-112.

Stich, H.F., Stich, W., Rosin, M.P. and Powrie, W.D. 1980b. Mutagenic activity of pyrazine derivatives: a comparative study with Salmonella typhimurium, Saccharomyces cerevisiae and Chinese hamster ovary cells. Food. Cosmet. Toxicol. 18: 581-584.

Stich, H.F., Rosin, M.P., Wu, C.H. and Powrie, W.D. 1981a. The action of transition metals on the genotoxicity of simple phenols, phenolic acids and cinnamic acids. Cancer Lett. (in press).

Stich, H.F., Rosin, M.P., Wu, C.H. and Powrie, W.D. 1981b. Clastogenicity of furans found in food. Cancer Lett. 13: 89-95.

Stich, H.F., Rosin, M.P., Wu, C.H. and Powrie, W.D. 1981c. A comparative genotoxicity study of chlorogenic acid (3-O-caffeoylquinic acid). Mutat. Res. 90: 201-212.

Stich, H.F., Stich, W. and Lam, P.P.S. 1981d. Potentiation and genotoxicity by concurrent application of compounds found in betel quid: arecoline, eugenol, quercetin, chlorogenic acid and Mn^{2+}. Mutat. Res. (in press).

Sugimura, T. 1979. Naturally occurring genotoxic carcinogens. In Naturally occurring carcinogens-mutagens and modulators of carcinogenesis. Edited by E.C. Miller, J.A. Miller, I. Hirono, T. Sugimura and S. Takayama. Jpn. Sci. Soc. Press, Tokyo/Univ. Park Press, Baltimore. pp. 241-261.

Uyeta, M., Taue, S. and Mazaki, M. 1981. Mutagenicity of hydrolysates of tea infusions. Mutat. Res. 88: 233-240.

Whiting, R.F., Wei, L. and Stich, H.F. 1980. Enhancement by transition metals of chromosome aberrations induced by isoniazid. Biochem. Pharmacol. 29: 842-845.

II.2 PAPERS PRESENTED/CONFERENCES

THE Ah GENE COMPLEX: A MODEL FOR STUDYING SPECIFIC INTERACTIONS OF CARCINOGENS AND TOXIC CHEMICALS

Allan B. Okey[a] and Daniel W. Nebert[b]

ABSTRACT

The Ah gene complex regulates the induction of cytochrome P_1-450 and at least two dozen associated drug-metabolizing enzyme activities. Chemicals which induce P_1-450 include many environmental contaminants such as polycyclic aromatic hydrocarbons (e.g. benzo(a)pyrene), PCBs, PBBs, dioxins and dibenzofurans. Induction is regulated by the Ah receptor which binds the inducing chemical in the cytoplasm, after which the inducer-receptor complex translocates into the nucleus where it stimulates production of specific mRNAs which code for P_1-450 and associated products of structural genes in the Ah complex. Enzymes regulated by the Ah gene complex are very important in both the "detoxication" and "toxification" of xenobiotics. The Ah gene model encompasses several specific sites at which interactions among xenobiotics may occur, especially at the Ah receptor level and at the cytochrome P_1-450 active site. Application of the Ah gene model in future evaluation of xenobiotic interactions is described.

[a] Division of Clinical Pharmacology, Department of Pediatrics, The Hospital for Sick Children, Toronto, Ontario M5G 1X8.

[b] Developmental Pharmacology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20205 USA.

LE COMPLEXE GENIQUE Ah: UN MODELE POUR L'ETUDE D'INTERACTIONS PARTICULIERES ENTRE CARCINOGENES ET TOXIQUES CHIMIQUES

Allan B. Okey[a] et Daniel W. Nebert[b]

RESUME

Le complexe génique Ah régularise l'induction du cytochrome P_1-450 et l'activité d'au moins deux douzaines d'autres enzymes apparentées métabolisant des substances chimiques. Les substances chimiques qui provoquent l'induction du P_1-450 comprennent un grand nombre de contaminants de l'environnement, tels les hydrocarbures aromatiques polycycliques (ex. le benzo(a)pyrène), les PCB, les PBB, les dioxines et les dibenzofuranes. L'induction est commandée par le récepteur Ah qui fixe la substance inductrice dans le cytoplasme, après quoi le complexe inducteur-récepteur est transporté dans le noyau où il stimule la production d'ARN messagers spécifiques qui "codifient" en fonction de la P_1-450 et d'autres substances apparentées de gènes structuraux du complexe Ah. Les enzymes contrôlées par le complexe Ah sont très importantes pour la "détoxification" et la "toxification" des xénobiotiques. Le modèle génique Ah englobe plusieurs sites spécifiques où il peut se produire des interactions entre xénobiotiques, surtout aux niveaux du récepteur Ah et du site actif du cytochrome P_1-450. On décrit l'application du modèle génique Ah à une évaluation future d'interactions xénobiotiques.

[a] Division de pharmacologie clinique, Département de pédiatrie, The Hospital for Sick Children, Toronto (Ontario) M5G 1X8.

[b] Developmental Pharmacology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20205 U.S.A.

OVERALL APPROACH TO THE COMBINED EFFECTS OF XENOBIOTICS

Given the vast number of existing xenobiotic chemicals and the number of physiologic systems upon which they act, the magnitude of potential interactions among xenobiotics is enormous. Far too little is known about the consequences of simultaneous exposure to multiple xenobiotics. Systematic research into the combined effects will need to proceed by testing specific hypotheses relating to potential interactions. However, these specific tests of combined effects must be founded on models which are able to be generalized, which then will allow rational allocation of research resources and which will be capable of unifying large amounts of data that could be generated by testing many xenobiotics combinations.

An experimental model currently exists which permits evaluation of several specific points at which multiple xenobiotic agents might interact. This paper summarizes the makeup of the Ah gene complex and cites selected examples which illustrate the utility of the complex as a tool or assessing xenobiotic interactions.

GENERAL NATURE OF CYTOCHROME P-450 MONOOXYGENASES AND COORDINATED ENZYMES

Many environmental pollutants and foreign compounds are known to be metabolized by the drug metabolizing enzyme systems.

Evidence is growing that metabolism to reactive intermediates by cytochrome P-450-mediated monooxygenases is a prerequisite for mutagenesis, carcinogenesis and toxicity caused by numerous drugs and environmental pollutants. These reactive intermediates probably bind covalently to cellular macromolecules. Most of this binding probably is random, but some may be non-random, i.e., specific binding which is dependent upon the chemical structures of the reactive intermediate and the cellular macromolecule. Among these types of covalent binding, there probably exists a very small amount of important binding of the ultimate carcinogen to its critical subcellular target, thereby initiating tumorigenesis. Two examples of apparent specific binding include that of benzo(a)pyrene 7,8-diol-9,10-epoxide to the ^{2}N-amino of guanine (Weinstein et al. 1976) and that of aflatoxin B_1 2,3-oxide to the ^{7}N of guanine (Essigmann et al. 1977).

The steady-state levels of these reactive electrophilic intermediates and, consequently, the rates at which they interact with the critical nucleophilic target are dependent upon a delicate balance between their generation and detoxication (Figure 1). Changes in the balance between "toxification" and "detoxication" in any particular tissue of an individual may therefore affect his risk of tumorigenesis or toxicity.

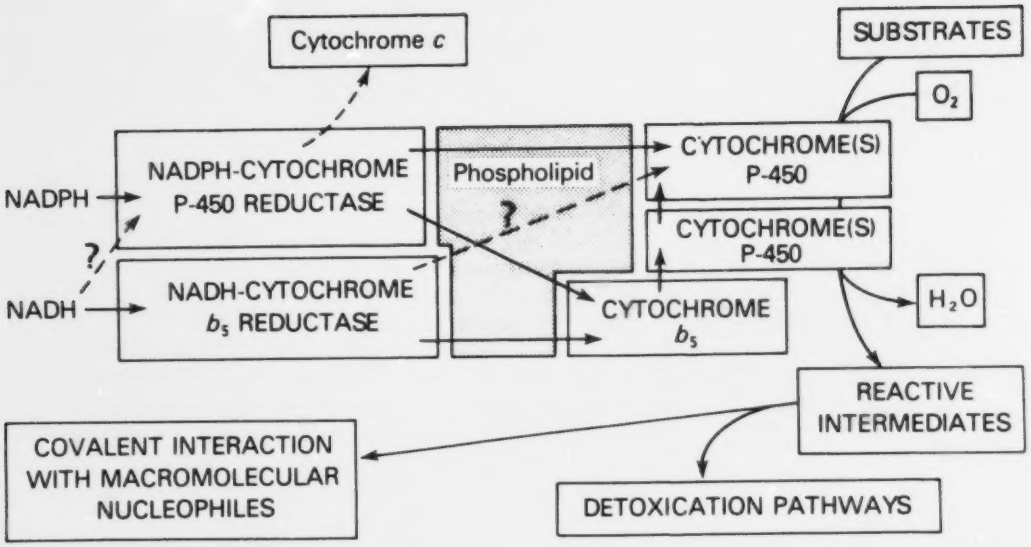


Figure 1. Scheme for the membrane-bound multicomponent monooxygenase system(s) and the various possibly important pathways for hydrophobic substrates. For any given substrate, the relative balance between metabolic activation and detoxication likely would differ among different tissues, strains, and species. Age, genetic expression, nutrition, hormone concentrations, diurnal rhythm, pH, subcellular compartmentalization of each enzyme, efficiency of DNA repair, and the immunological competence of the animal -- all may be important factors affecting this balance. From Nebert (1981). Reproduced with permission from the National Institute of Environmental Health Sciences.

MULTIPLE FORMS OF CYTOCHROME P-450

Endogenous P-450

Several control forms of P-450 have been purified to apparent homogeneity (see Nebert et al. 1981). There is indirect evidence for at least four forms from rat liver and seven forms from mouse liver. In all cases, these forms of apparently constitutive P-450 are able to metabolize many xenobiotics and numerous endogenous substrates with varying degrees of overlapping substrate specificities.

One may speculate that endogenous forms of P-450 may carry out more specific metabolic reactions than induced forms of P-450 because many biosynthetic and catabolic processes of steroids and biogenic amines may be critical to important life functions. The ability of constitutive forms of P-450 to metabolize xenobiotics might be viewed as fortuitous, yet could be important for survival in a chemically adverse environment. It would be advantageous to the organism if endogenous P-450 could metabolize, to some extent, a toxic chemical suddenly added to the environment--until forms of P-450 capable of metabolizing the toxic chemical more effectively are induced.

How many forms of endogenous P-450 are there and how are they regulated? In view of the dozens of distinct catalytic activities described for normal body substrates such as steroids, indoles, biogenic amines, prostaglandins and thyroxine, it seems likely that ultimately more than 10 and probably less than 100 endogenous forms of P-450 (microsomal and mitochondrial) will be found.

Induced P-450

More than 10 years ago (Conney 1967), P-450 inducers were classified as being of two major types--"3-methylcholanthrene-like" or "phenobarbital-like"--although it was also well known that more than 200 drugs, carcinogens, other environmental pollutants and steroids induce their own metabolism and/or that of other compounds via P-450 induction. This classification appears to have originated from research interest in cancer in the 1950s (Conney et al. 1957) and in barbiturate tolerance in the early 1960s (Remmer and Siegert 1962). Polycyclic hydrocarbon carcinogens (3-methylcholanthrene) and barbiturates (phenobarbital) were each found empirically to induce a variety of drug-metabolizing enzyme activities, and these two "classes" of inducers appeared to be very different and, in fact, appeared additive when administered together (Conney 1967).

As more sophisticated techniques for separating forms of P-450 and for assaying an increased number of monooxygenase activities have become available these past several years, an increasing number of inducing compounds appear neither precisely 3-methylcholanthrene-like nor precisely phenobarbital-like.

The number of different forms of induced P-450 is not known. Until now, the general consensus has been that fewer than one dozen forms of induced P-450 exist and that overlapping substrate specificity accounts for all diversity seen when thousands of different chemicals are metabolized by the monooxygenase system. At the other extreme, it has been postulated (Nebert 1979b) that organisms have the genetic capacity to induce as many distinct forms as there are inducers of P-450. Possessing the genetic capacity to form hundreds or thousands of different P-450 species does not mean that all of them would exist at any one time. At any given moment, an organism may be exposed to significant concentrations of perhaps only 10 or 20 important inducers, so that 10 or 20 forms of induced P-450 would exist in quantities sufficient to be detected. At a later time, the same organism, because of changes in its environment, may develop a different profile of detectable forms of induced P-450. Such a hypothetical situation is similar to immunoglobulin formation in response to various antigens encountered by an organism. Further work is needed to confirm or disprove this hypothesis about induced forms of P-450.

THE *Ah* GENE COMPLEX: REGULATION OF CYTOCHROME P_1-450

GENERAL DESCRIPTION: THE PLEIOTYPIC RESPONSE

The murine *Ah* locus regulates the induction by polycyclic aromatic compounds (such as 3-methylcholanthrene, β-naphthoflavone, benzo(a)pyrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin [TCDD]) of numerous drug-metabolizing enzyme activities in virtually all tissues (Nebert and Jensen 1979). The *Ah* complex is viewed as a combination of regulatory, structural, and probably temporal genes which may or may not be linked. The complex is illustrated diagramatically in Figure 2.

The first events during the induction process are believed to be passive transfer of xenobiotic inducers across the cell membrane (Nebert and Bausserman 1971) and the highly specific binding to the cytosolic *Ah* receptor (Poland *et al.* 1976; Okey *et al.* 1979), regarded as the major regulatory gene product. Following the apparent translocation of the inducer-receptor complex into the nucleus (Okey *et al.* 1980), the pleiotypic response includes the activation of several structural genes. The induction of several drug-metabolizing enzymes (structural gene products) leads to a delicate balance between increased formation of reactive intermediates and detoxication.

Response to aromatic hydrocarbons was first characterized in the C57BL/6 inbred strain (B6, responsive, Ah^b), and DBA/2 was the first nonresponsive mutant inbred strain characterized (D2, nonresponsive, Ah^d). The Ah^b/Ah^d heterozygote is responsive, indicating a Mendelian autosomal dominant trait. The new monooxygenase activities can be induced in nonresponsive mice by a dose of TCDD 12-18 times larger than that needed in responsive inbred strains (Poland *et al.* 1974). The response to high doses of TCDD suggests that nonresponsive mice have a

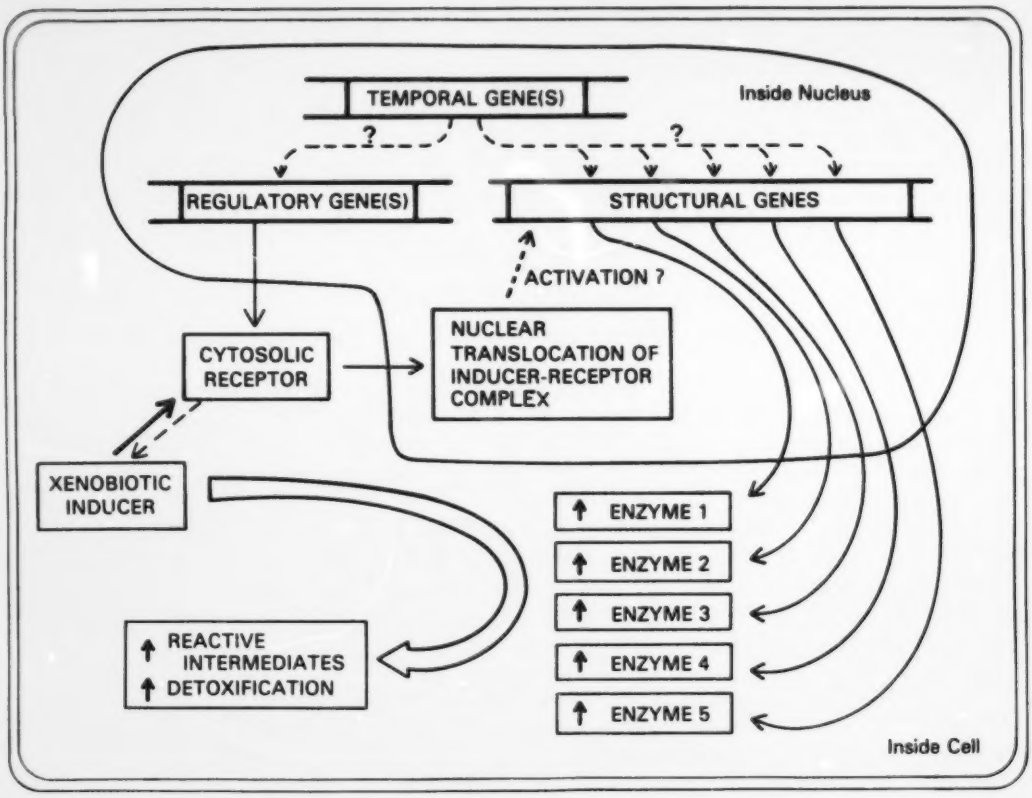


Figure 2. Diagram of a cell depicting the Ah complex. Major product of the regulatory gene is the cytosolic receptor. Various induced enzymes represent products of different structural genes of the Ah complex. Although a temporal gene or genes are postulated within the Ah complex, it presently is not clear whether this temporal control affects regulatory or structural genes. Most inducers are metabolized by various induced enzymes to reactive intermediates and innocuous products. Depending on half-life of the intermediate, rate of formation of reactive intermediates, and rate of conjugation and other means to detoxify the intermediate, important covalent binding may occur in the same cell in which metabolism occurred or in some distant cell and may be associated with initiation of cancer or toxicity. From Nebert (1980a). Reproduced with permission from the U.S. Government Printing Office.

defective Ah receptor, but intact structural genes. This difference in sensitivity for any Ah-locus-associated inducer has been found for several monooxygenase activities in virtually every tissue of the mouse (Nebert and Jensen 1979).

It should be noted that the Ah locus does not reflect a single-gene difference but rather is much more complicated (Nebert and Jensen 1979). Regulation alone must involve a minimum of six alleles and two loci, and structural gene mutants appear to exist as well as regulatory gene mutants.

MULTIPLE STRUCTURAL GENE PRODUCTS: INDUCED P-450s AND ASSOCIATED ENZYME ACTIVITIES

The induction of at least two dozen monooxygenase activities appears to be under the control of the same Ah receptor (Table 1). The substrates listed in Table 2 are highly variable in size and shape, and the question was raised (Nebert et al. 1973) whether these induced activities represent relatively non-specific metabolism by a single form of polycyclic aromatic-induced P-450 or more specific metabolism carried out by a large family of individual enzymes. Although most laboratories have been hesitant to accept the possibility, considerable evidence exists that more than one single form of "cytochrome P-448" is induced by 3-methylcholanthrene treatment (Nebert et al. 1981).

Other inducible enzymes that are not monooxygenases but which appear to be metabolically coordinated and associated with the Ah^b allele include microsomal UDP glucuronosyltransferase and cytosolic reduced NAD(P):menadione reductase (Nebert and Jensen 1979). Cytosolic ornithine decarboxylase becomes markedly enhanced early during the induction process and is also associated with presence of the Ah receptor (Nebert et al. 1980).

THE Ah RECEPTOR: SPECIFIC BINDING OF XENOBIOTIC CHEMICALS TO THE REGULATORY GENE PRODUCT OF THE Ah LOCUS

The increase in monooxygenase activities caused by "3-methylcholanthrene-like" chemicals is a true inductive response, i.e. it requires de novo synthesis of specific hemoproteins (Haugen et al. 1976). Induced cytochrome P_1-450, then, is a major structural gene product of the Ah complex.

Some years ago it was postulated (Nebert and Bausserman 1970; Poland et al. 1974) that a regulatory gene in the Ah complex might produce a protein which functions as the receptor for chemical inducers of the "3-methycholanthrene-type". Recently we have characterized such a receptor which is the major regulatory gene product of the Ah complex (Okey et al. 1979). The Ah receptor was detected using [^{3}H]TCDD. TCDD is an extremely potent inducer of cytochrome P_1-450 and other activities

Table 1. All induced monooxygenase 'activities' known to be associated with *Ah*^{b} allele[α].

Aryl hydrocarbon (benzo[a]pyrene) hydroxylase (AHH)
7-Ethoxycoumarin O-demethylase
p-Nitroanisole O-demethylase
Dimethylaminoazobenzene N-demethylase
Zoxazolamine 6-hydroxylase
Acetylarylamine (2-acetylaminofluorene) N-hydroxylase
Biphenyl 2-hydroxylase
Biphenyl 4-hydroxylase
Naphthalene monooxygenase
Acetaminophen N-hydroxylase
p-Chloroacetanilide N-hydroxylase
Phenacetin O-deethylase
Ethoxyresorufin O-deethylase
Aflatoxin B_1 4-hydroxylase
Amaranth azoreductase
β-Naphthoflavone metabolism to unidentified products
α-Naphthoflavone metabolism to unidentified products
Ellipticine metabolism to unidentified products
Lindane metabolism to unidentified products
Nitridazole metabolism to unidentified products
Caffeine metabolism to unidentified products
Theophylline metabolism to unidentified products
Theobromine metabolism to unidentified products

[α] All basal enzyme activities so far tested, with the exception of biphenyl 2-hydroxylase, are preferentially inhibited by metyrapone *in vitro*, indicating an association of these catalytic activities with one or more forms of P-450 other than P_1-450. All polycyclic aromatic compound-induced enzyme activities so far tested, plus basal biphenyl 2-hydroxylase activity, are preferentially inhibited by α-naphthoflavone, more so than metyrapone *in vitro*, indicating an association of these catalytic activities with cytochrome P_1-450, P-488 (or other inducible form of P-450).

Table 2. Specific [^{3}H]TCDD binding to B6 cytosol receptor in the presence of compounds that have varied potencies for hepatic cytochrome P_1-450 induction.

Compound tested	Potency as an inducer of P_1-450	Binding as percent of control[α]	
		Sucrose density gradient assay	Dextran-charcoal assay
Bilirubin	0	100	140
Hematin	0	100	115
Cholesterol	0	95	115
Cholic acid methyl ester	0	95	115
Phenobarbital	±	110	150
Pregnenolone-16α-carbonitrile	0	100	115
Dexamethasone	0	110	155
β-Methasone	0	90	100
Progesterone	0	110	185
Estradiol-17β	0	95	100
Dihydrotestosterone	0	100	120
p,p'-DDT	+	35	40
α-Naphthoflavone	++	70	120
β-Naphthoflavone	+++	25	110
Benzo[a]pyrene	+++	0	135
3-Methylcholanthrene	+++	0	175
Benz[a]anthracene	+++	0	85
TCDD	++++	0	165
2,3-Dichlorodibenzo-ρ-dioxin	+	45	85
1,2,4-Trichlorodibenzo-ρ-dioxin	++	45	70
1,2,4,7,8-Hexachlorodibenzo-ρ-dioxin	+++	15	205
Octachlorodibenzo-ρ-dioxin	0	100	165

[α] Specific binding was determined by sucrose density gradient analysis after dextran-charcoal treatment using 10 nM [^{3}H]TCDD. Each compound tested (10 nM) was added to cytosol 15 min before [^{3}H]TCDD, using ethanol as a vehicle for steroids or polycyclic aromatic hydrocarbons. Bilirubin and hematin were added as aqueous solutions, and ρ-dioxane was used as a solvent for all dibenzo-ρ-dioxins. An equal volume (10 to 20 μL/mL) of the appropriate solvent was added to cytosol in control tubes. The "dextran-charcoal assay" represents [^{3}H]TCDD remaining in the cytosol after dextran-charcoal treatment and without separation of specific binding peaks by sucrose density gradient centrifugation.

associated with the Ah locus (Nebert et al. 1981) and is a uniquely useful probe in elucidating the mechanism of induction.

The initial event in P_1-450 induction by "3-methylcholanthrene-like" inducers is binding of the inducer to a cytoplasmic receptor protein. Since TCDD also binds to many cytosolic proteins other than the induction-receptor, it is necessary to have an assay by which specific receptor binding can be distinguished from non-specific, non-saturable binding. (The receptor accounts for less than 1% of all [^{3}H]TCDD bound within hepatic cells.) The only method we have found which reliably distinguishes specific from non-specific binding is sucrose density gradient centrifugation after charcoal-dextran treatment. With this procedure we have characterized a cytosolic binding protein which fulfills all the criteria expected of a receptor regulating the Ah complex.

Figure 3 illustrates the specific high-affinity binding of [^{3}H]TCDD to hepatic cytosol from C57BL/6 mice; the high-affinity binding peak is displaced by non-labelled TCDD or 3-methylcholanthrene, but not by phenobarbital (which induces different forms of P-450).

Table 2 further illustrates that the receptor detected by sucrose density gradient analysis is specific for compounds which induce cytochrome P_1-450. In general properties, the receptor is principally protein, highly thermolabile and stabilized in the presence of glycerol. The concentration of cytosolic receptor sites in C57BL/6N mice is about 5,500 per hepatic cell (~ 50 fmol/mg cytosol protein) with an apparent K_d for [^{3}H]TCDD binding estimated to be approximately 0.7 nM (Okey et al. 1979).

It can be shown that specific [^{3}H]TCDD binding is distinctly associated with the Ah^b allele. The receptor peak is present in hepatic cytosol from the prototype responsive strain, C57BL/6N, but is not detectable in the prototype nonresponsive strain DBA/2N (Figure 4). Further genetic analysis using offspring from the B6D2F_1 x D2 backcross reveals that the cytosolic peak is present in phenotypically responsive Ah^b/Ah^d heterozygotes, but not in the nonresponsive Ah^d/Ah^d homozygote (Figure 4). Thus, mice having the Ah^b allele possess the receptor in liver cytosol (and P_1-450 is "inducible") whereas Ah^d/Ah^d individuals lack the detectable receptor and thus P_1-450 induction is insensitive to 3-methylcholanthrene-like inducers.

Although specific binding to the cytosolic receptor is an essential first step in the induction process, cytosolic binding alone seems insufficient to activate structural genes. If [^{3}H]TCDD is injected into a mouse known to have the Ah^b allele, a peak of [^{3}H]TCDD binding can be demonstrated in nuclear extracts (Figure 5); this binding peak is very small or absent in nonresponsive DBA/2N mice and in nonresponsive Ah^d/Ah^d backcross homozygotes.

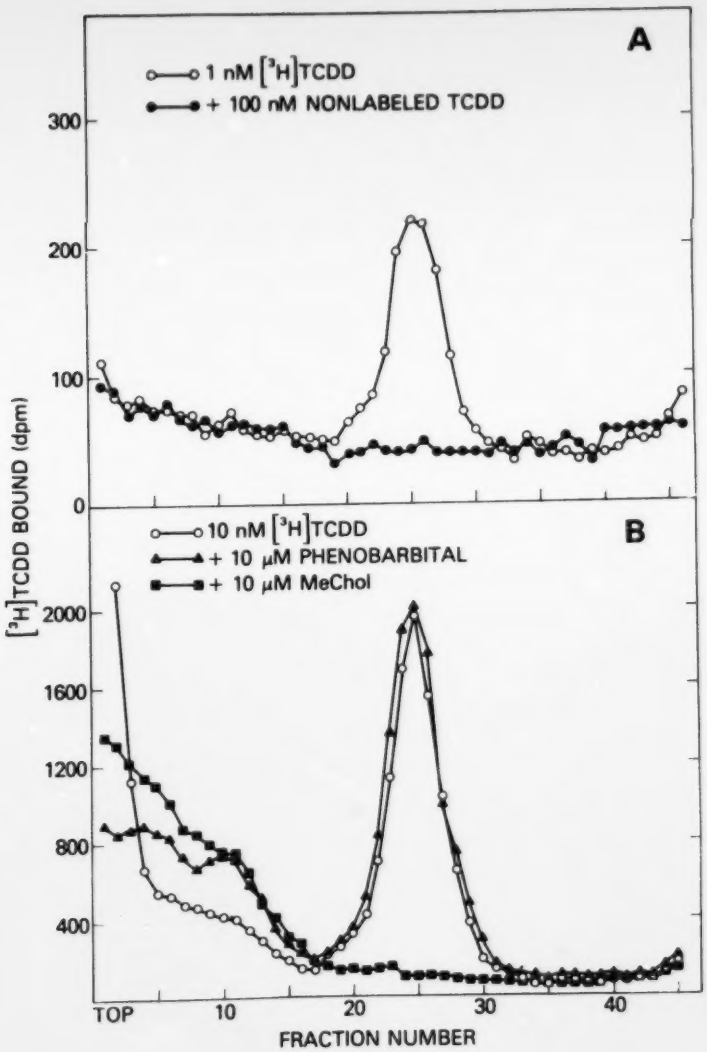


Figure 3. Detection of specific [^{3}H]TCDD binding of a component from B6 hepatic cytosol. (A)Cytosol (1 mg protein/mL) from an eight-week-old B6 male was incubated with 1 nM [^{3}H]TCDD in the absence of competitor (o—o) or in the presence of 100 nM nonlabeled TCDD (●—●) for 1 h at 4°C. After dextran-charcoal treatment, gradients were centrifuged and fractionated. (B) Elimination of specific binding peak by 3-MC but not by phenobarbital. Cytosol (5 mg protein/mL) was incubated with 10 nM [^{3}H]TCDD in the absence of competitor (o—o), and in the presence of 10 nM 3-MC (■—■) or 10 μM phenobarbital (▲—▲). From Okey et al. (1979). Reproduced with permission from the American Society of Biological Chemists.

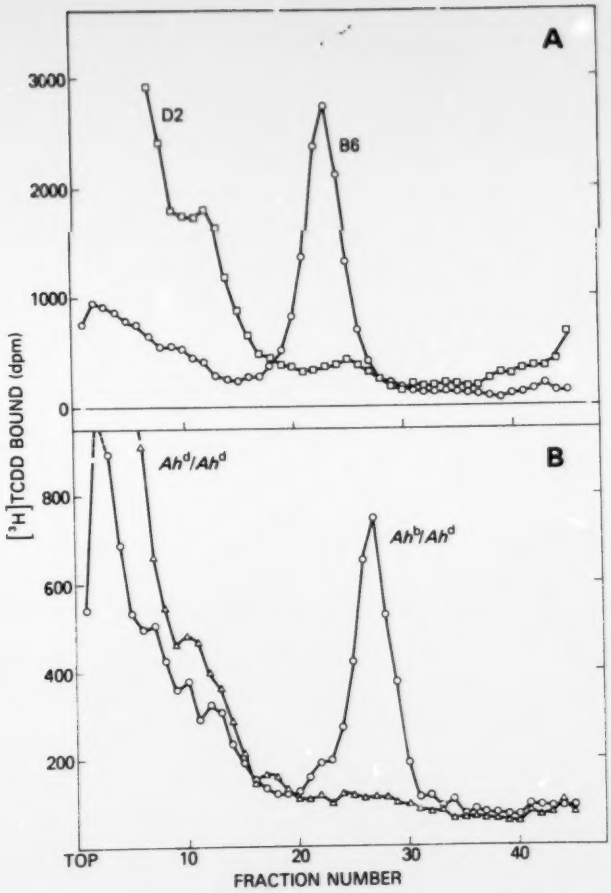


Figure 4. Genetic differences in hepatic cytosolic receptor. (A) Hepatic cytosolic fractions (5 mg protein/mL) from responsive B6 or nonresponsive D2 inbred mice were incubated at 4°C (with 10 nM [^{3}H]TCDD) and analyzed as usual by sucrose density gradient separation after dextran-charcoal treatment. (B) Hepatic cytosolic fractions from a phenotypically responsive Ah^b/Ah^d heterozygote (3.7 mg protein/mL) were incubated at 4°C (with 10 nM [^{3}H]TCDD) and analyzed as usual. The binding peak in cytosol from the responsive mouse was eliminated by incubation in the presence of 100 nM nonlabelled TCDD (data not shown), thus confirming its limited capacity. These progeny from the $B6D2F_1$ x D2 backcross had been phenotyped by the zoxazolamine paralysis test more than one week before this receptor assay. From Okey et al. (1979). Reproduced with permission from the American Society of Biological Chemists.

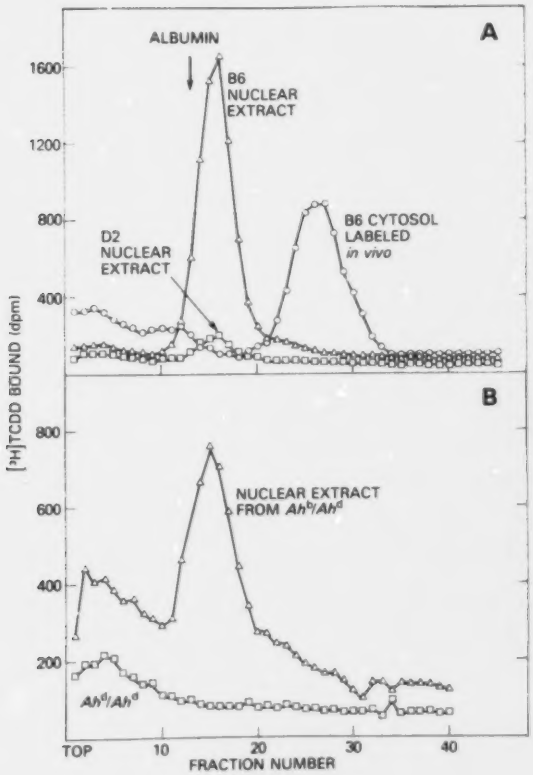


Figure 5. Genetic differences in the nuclear binding of [^{3}H]TCDD in vivo. (A) Nuclear extracts (approximately 5 mg protein/mL) from responsive B6 and nonresponsive D2 liver were treated with dextran-charcoal and then centrifuged on sucrose density gradients prepared in HEDG buffer containing 0.4 M KCl.B6 cytosol (labeled in vivo, 15 mg protein/mL) was treated with dextran-charcoal and centrifuged as usual on a gradient prepared in HEDG buffer without KCl. (B) Hepatic nuclear extracts from a responsive Ah^b/Ah^d and a nonresponsive Ah^d/Ah^d individual from the B6D2F$_1$ x D2 backcross. The extracts (6 mg protein/mL) after dextran-charcoal treatment were centrifuged on gradients prepared in HEDG buffer containing 0.4 M KCl. The B6 and D2 mouse had each received 2 µg of [^{3}H]TCDD (approximately 0.3 µmol/kg body weight) and were killed 2 h later. The backcross animals had each received 5 µg of [^{3}H]TCDD (about 0.75 µmol/kg body weight) and were killed 3 h later. These backcross mice had been phenotyped more than one week earlier. From Okey et al. (1979). Reproduced with permission from the American Society of Biological Chemists.

Nuclei isolated directly from livers of untreated mice do not contain specific binding. Accumulation of receptor in the nucleus is accompanied by a decrease in the cytosol receptor (Okey et al. 1980). This and other evidence indicates that inducing chemicals first bind to the receptor in the cytoplasm and that this initial step is followed by translocation of the inducer-receptor complex into the nuclear compartment. Recent experiments with responsive hepatoma cell lines in culture reveal that receptor translocation into the nucleus is temperature-dependent, suggesting that some form of "activation" or "transformation" of receptor must occur before nuclear translocation is possible (Okey et al. 1980).

Once in the nucleus, it is believed that the inducer-receptor complex binds to some recognition ("acceptor") site(s) associated with the Ah locus. At present, there is little known about the precise mechanism by which presence of the inducer-receptor complex in the nucleus stimulates transcription of specific mRNAs coding for structural gene products associated with the Ah gene complex.

The overall receptor mechanism described is strikingly similar to that which mediates biological effects of steroid hormones. Although the Ah receptor shares many structural and functional features in common with receptors for steroid hormones, it should be emphasized that reciprocal competition studies rule out the possibility that [^{3}H]TCDD is binding to and acting through a known steroid-hormone receptor.

SOME CONSEQUENCES OF GENETIC DIFFERENCES AT THE Ah LOCUS

The previous sections have described general functions of cytochrome P-450-mediated monooxygenases and have emphasized the role of the Ah receptor in the regulation of cytochrome P_1-450. The Ah locus is an experimental model system that has provided several good examples of a delicate balance in the interaction between genetic and environmental factors in the etiology of cancer, drug toxicity, and birth defects. We will now summarize briefly a few of the toxic phenomena that have been shown to be associated with the Ah gene complex in the mouse.

As indicated in Table 3, genetically responsive Ah^b/Ah^b or Ah^b/Ah^d mice often are at higher risk for toxicity or tumorigenesis than are nonresponsive Ah^d/Ah^d individuals. Under certain conditions, however, nonresponsive mice are at higher risk than equivalently treated responsive mice. The route of administration is the major factor determining which genotype is at higher risk.

When a carcinogen or other toxic drug is placed in relatively direct contact with the tissue being studied, the genetically responsive Ah^b/Ah^b or Ah^b/Ah^d mouse is at increased risk for developing a tumor or toxicity in that tissue, compared with a nonresponsive Ah^d/Ah^d mouse receiving the same dose of xenobiotic; this concept is illustrated in Figure 6. On the other hand, if the malignancy or toxicity is found at

Table 3. Summary of toxicity and tumorigenesis in the mouse associated with the Ah locus (Nebert 1981).

Individual at increased risk	Tumor or toxicity	Route of administration	Chemical
Ah^b/Ah^b and	Skin inflammation	Topical	7,12-Dimethylbenzo[a]anthracene
Ah^b/Ah^d	Fibrosarcomas	Subcutaneous	3-Methylcholanthrene or BP
	Pulmonary tumors	Intratracheal	3-Methylcholanthrene or BP
	In utero fetal toxicity	Intraperitoneal	BP, 3-Methylcholanthrene, 7,12-Dimethylbenzo[a]anthracene
	Primordial oocyte depletion	Intraperitoneal	7,12-Dimethylbenzo[a]anthracene, 3-Methylcholanthrene, BP
	Epidermal carcinoma	Topical	BP
	Cleft palate in fetus	Intraperitoneal	2,3,7,8-Tetrachlorodibenzo-p-dioxin
	Experimental porphyria	Intraperitoneal	Chlorinated aromatic compounds
Ah^d/Ah^d	Lymphoma, lymphosarcoma	Intraperitoneal	7,12-Dimethylbenzo[a]anthracene
	Bone marrow toxicity	Oral	BP
	Leukemia	Subcutaneous	3-Methylcholanthrene
	Leukemia	Oral	BP

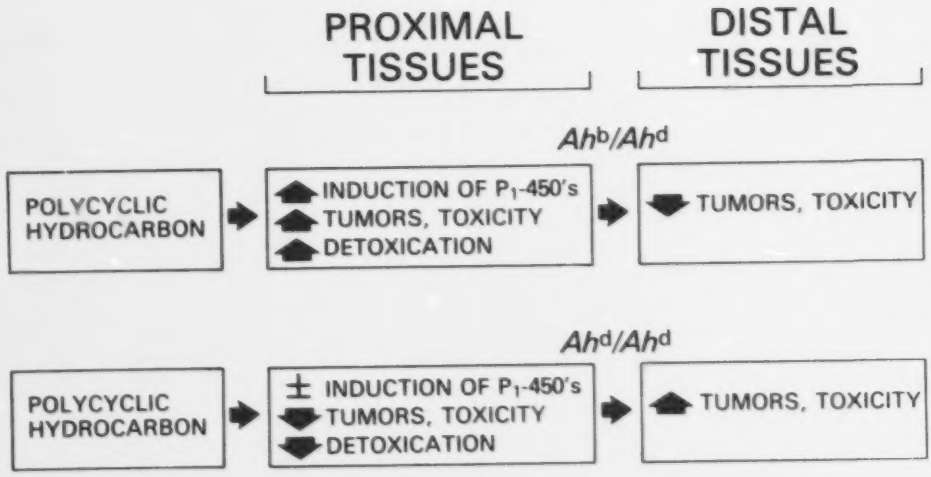


Figure 6. Illustrated scheme indicating that the genetically responsive $\underline{Ah}^b/\underline{Ah}^d$ individual (top) is at increased resk for tumors or toxicity at sites in direct contact with the xenobiotic. The nonresponsive $\underline{Ah}^d/\underline{Ah}^d$ individual (bottom) is at increased risk for tumors or toxicity at distant sites, due to decreased "detoxication" in many tissues of the body. From Nebert (1980b). Reproduced with permission from Academic Press, Inc.

a site distant from the administered drug, the Ah^d/Ah^d mouse is at increased risk, compared with an Ah^b/Ah^b or Ah^b/Ah^d individual receiving the same dose of xenobiotics. In this latter case, the responsive animal is probably protected primarily by the "first-pass" effect (also termed "presystemic drug elimination"; Routledge and Shand 1979). Fundamentally, presystemic elimination reflects the metabolism and excretion of a drug before it reaches its site of action.

Enhancement of presystemic elimination can be achieved experimentally by pretreating responsive animals with any of several chemicals (benzo(a)pyrene, β-naphthoflavone, phenobarbital, DDT) which induce monooxygenases and associated enzymes. If carcinogens such as benzo(a)pyrene or 7,12-dimethylbenz(a)anthracene are given orally to preinduced animals, the pretreatment can substantially protect against neoplasia in lung, skin and forestomach (Wattenberg 1978), breast (Silinskas and Okey 1975) and bone marrow (Nebert 1981).

Prevention of neoplasia by preexposure of responsive animals to monooxygenase-inducing chemicals might be considered a benevolent interaction among two or more xenobiotics. It is obvious from data in Table 3, however, that treatments which reduce tumor risk at one site may increase the risk of neoplasia or toxicity at other sites. Induction of cytochrome P_1-450 represents a "double-edged sword"; therefore, in all cancer and toxicity experiments, not only the identity of interacting chemicals, but also the genetic background, dose, route of administration, as well as the tissue in which toxicity of malignancy develops, must be considered during interpretations.

EVIDENCE OF THE Ah LOCUS IN HUMANS

The discovery of the genetic regulation of P_1-450-mediated drug metabolism in mice signals an area potentially of great importance in evaluating susceptibility of individual humans to drugs and environmental chemicals. Kellermann and associates (1973) reported that aryl hydrocarbon hydroxylase (AHH) induction in human lymphocytes exhibits a trimodal distribution in the population ("low", "intermediate", and "high" inducibility), consistent with a hypothesis of two alleles at a single locus. Furthermore, they concluded that a person having the "intermediate" phenotype has a 16-fold increased risk and a person having the "high" phenotype, a 36-fold increased risk of developing bronchogenic carcinoma compared with persons having the "low inducibility" phenotype. Because of the variability of mitogen-stimulated lymphocyte assays employed however, it has been difficult to confirm the monogenic hypothesis.

There is little doubt that heritable variation in P_1-450 (AHH) inducibility occurs in man (Nebert and Jensen 1979). Experimental difficulties make it presently impossible to be certain whether induction in humans is controlled by one or more loci, i.e. polygenic.

In spite of the shortcomings of the lymphocyte assay, a growing list of clinical disorders (Table 4) appear to be associated with the human Ah locus. Evidence suggests that a high ratio of P_1-450 to other forms of P-450 exists in many, if not all, extrahepatic tissues in vivo. This also appears to be the case in cultured lymphocytes, monocytes, pulmonary macrophages, and even skin fibroblasts. Until one can increase the range of inducibility of AHH activity, and/or decrease the magnitude of daily variability of "control" activity, AHH induction in cultured mitogen-activated lymphocytes or any similar test system cannot be used as a promising biochemical marker for predicting who is at risk for bronchogenic carcinoma, aplastic anemia, leukemia, or various other types of environmentally-caused toxicity or malignancy.

APPLICATION OF THE Ah GENE MODEL IN TOXICOLOGY: SELECTED EXAMPLES

As described throughout this paper, cytochrome P-450-mediated enzymes play central roles in "toxification" and "detoxication" of a wide variety of xenobiotic chemicals. A genetic difference in cytochrome P_1-450 induction has been shown to be an important determinant of the response of experimental animals to xenobiotic agents; similar genetic differences are likely in the human population. Figure 7 integrates the concept of P_1-450 induction in genetically responsive cells with the consequences which this induction may have when the cell is later exposed to another xenobiotic.

If nature is kind, there will be a limited number of mechanisms and pathways by which chemicals are toxic, become toxic, or potentiate (or ameliorate) the toxicity of other agents. What properties does the Ah gene model possess which makes it a valuable tool for evaluating xenobiotic interactions and the consequences of such interactions?

The model describes specific sites at which xenobiotic chemicals are likely to interact, especially at the cytosolic Ah receptor level and at the cytochrome P_1-450 active site; these are common points at which many xenobiotic agents are likely to converge.

CHEMICALS KNOWN TO INTERACT VIA COMPETITION FOR CYTOSOLIC Ah RECEPTOR

Table 2 lists several xenobiotic chemicals which are known to compete with [^{3}H]TCDD for binding to the cytosolic Ah receptor from mouse liver. Effective competitors include the carcinogenic hydrocarbons benzo(a)pyrene, benz(a)anthracene and 3-methylcholanthrene and various congeners of chlorodibenzo-p-dioxins. The Ah receptor also has been reported to bind toxic congeners of dibenzofuran and azoxybenzene (Poland et al. 1979).

Recently the Ah receptor has been shown to bind several polychlorinated biphenyl (PCB) congeners (Bandiera et al. 1982). As illustrated in Figure 8, PCB congeners which are "methylcholanthrene-

Table 4. Human disorders that appear to be associated with the Ah locus (Nebert 1981).

Disorder	Association with high or low AHH inducibility
Malignancy	
Bronchogenic carcinoma	High[α]
Bronchogenic carcinoma	No association found
Laryngeal carcinoma	High[β]
Cancer of oral cavity	High[β]
Cancer of renal pelvis or ureter	No association found
Cancer of urinary bladder	No association found
Acute leukemia of childhood	Low[α]
Toxicity	
Zoxazolamine-induced fatal hepatic necrosis	Unknown[γ]
Earlier onset of menopause among cigarette smokers	Unknown[γ]
Infertility among cigarette smokers	Unknown[γ]
Acetaminophen-induced diffuse bilateral cataracts	Unknown[γ]

[α] Consistent with genetic data from inbred strains of mice.

[β] Studies of these disorders in mice have not been specifically carried out, but the human data are consistent with what is known about environmental carcinogens and their effect on local and distant tissue sites in genetically "Ah-responsive" and "Ah-nonresponsive" mice.

[γ] Genetically responsive mice are at increased risk for these disorders. In retrospect (or in studie to be designed in the future), it would have been (or would be) of interest to know the Ah phenotyp of afflicted clinical patients.

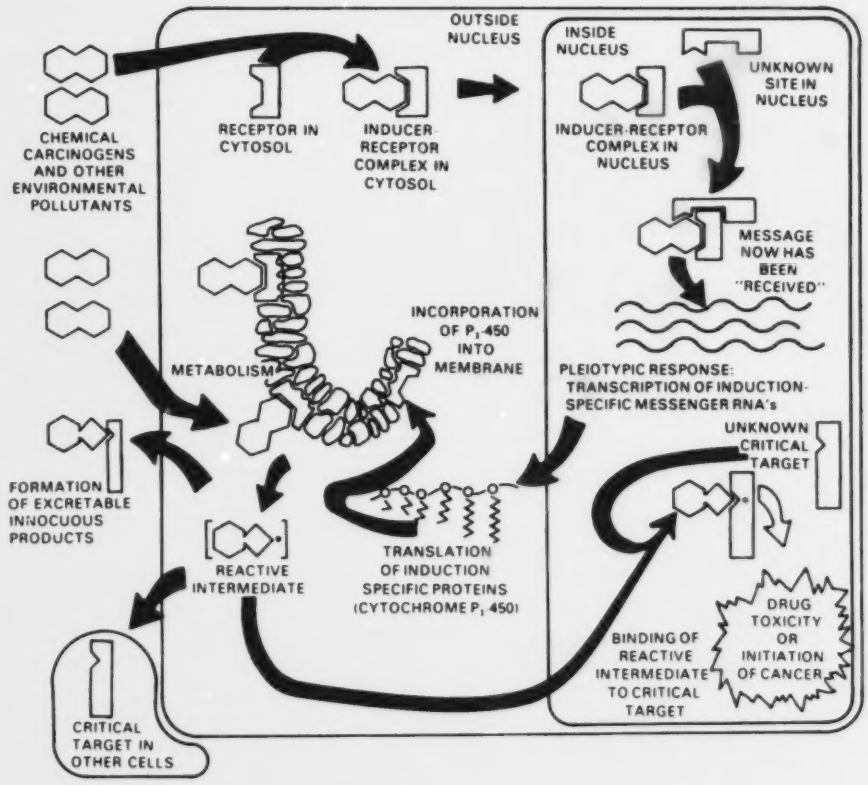

OUTSIDE NUCLEUS
INSIDE NUCLEUS
CHEMICAL CARCINOGENS AND OTHER ENVIRONMENTAL POLLUTANTS
RECEPTOR IN CYTOSOL
INDUCER-RECEPTOR COMPLEX IN CYTOSOL
INDUCER-RECEPTOR COMPLEX IN NUCLEUS
UNKNOWN SITE IN NUCLEUS
MESSAGE NOW HAS BEEN "RECEIVED"
INCORPORATION OF P1-450 INTO MEMBRANE
METABOLISM
PLEIOTYPIC RESPONSE: TRANSCRIPTION OF INDUCTION-SPECIFIC MESSENGER RNA's
UNKNOWN CRITICAL TARGET
FORMATION OF EXCRETABLE INNOCUOUS PRODUCTS
REACTIVE INTERMEDIATE
TRANSLATION OF INDUCTION SPECIFIC PROTEINS (CYTOCHROME P1 450)
DRUG TOXICITY OR INITIATION OF CANCER
BINDING OF REACTIVE INTERMEDIATE TO CRITICAL TARGET
CRITICAL TARGET IN OTHER CELLS

Figure 7. Diagram of a cell and the hypothetical scheme by which a cytosolic receptor, a product of the regulatory Ah gene, binds to inducer. The ultimate "pleiotypic response" includes greater amounts of cytochrome P_1-450 (and numerous other forms of P-450 still being characterized), leading to enhanced steady-state levels of reactive intermediates, which are associated with genetic increases in drug toxicity, teratogenesis or chemical carcinogenesis. Depending upon the half-life of the reactive intermediate, important covalent binding may occur in the same cell in which metabolism took place or in some distant cell. Although the "unknown critical target" is illustrated here in the nucleus, there is presently no experimental evidence demonstrating unequivocally the subcellular location of a "critical target(s)" required for the initiation of drug toxicity or cancer or, for that matter, whether the "target" is nucleic acid or protein. From Nebert (1979b). Reproduced with permission from Dr. W. Junk Publishers.

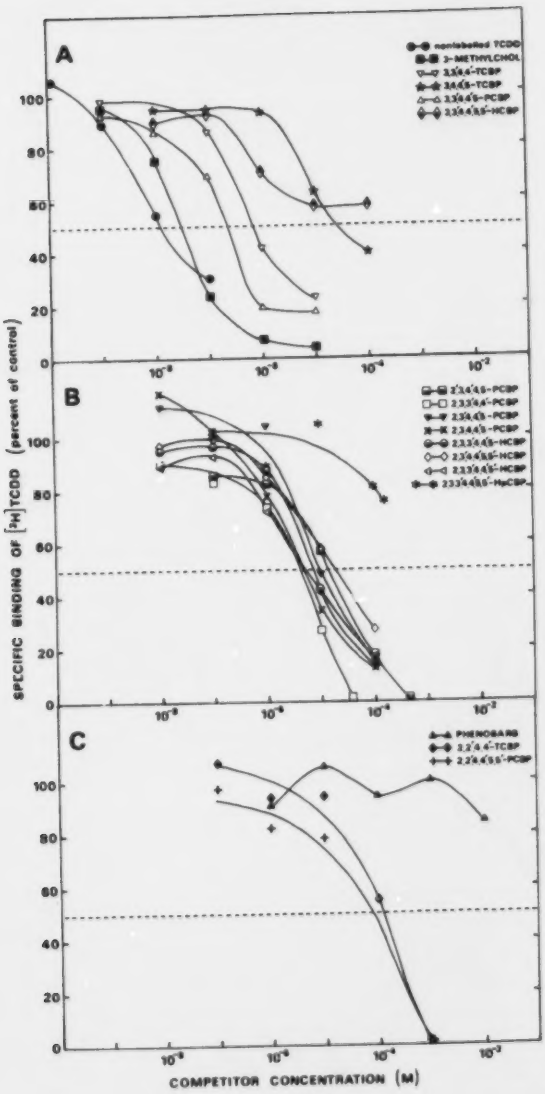


Figure 8. Comparative competition by chlorinated biphenyl congeners for binding to the Ah receptor. (A) Competition by "pure MC-like inducers". Rat hepatic cytosol (5 to 6 mg protein/mL) was incubated with 10 nM ^{3}H-TCDD in the presence of varying concentrations of nonlabeled TCDD, MC, or PCB congeners. (B) Competition by "mixed-type inducers" using the same conditions as in (A); (C) Competition by "PB-like inducers" using the same conditions as in (A). From Bandiera et al. (1982).

like" inducers are the most effective in competing with [^{3}H]TCDD for binding to the Ah receptor; "mixed-inducer" type PCBs and "phenobarbital-like" PCBs also interact with the receptor, but with lower affinity than that of "methylcholanthrene-like" PCBs.

It can be seen that most xenobiotic chemicals which bind with high affinity to the Ah receptor are highly toxic and/or carcinogenic. Poland et al. (1979) have proposed that the toxicity of chlorinated aromatic hydrocarbons, in general, is directly mediated through their binding to the Ah receptor.

In another application, the Ah receptor was employed to determine the total biological activity present in such complex environmental samples as fly ash from municipal incinerators (Hutzinger et al. 1981). In that study, it was found that the total biological activity of fly ash was only partially accounted for by the sample's content of 2,3,7,8-TCDD. Receptor-competition assays, then, can be used to evaluate total biological activity in complex mixtures without the need for immediate identification of specific components.

CHEMICALS KNOWN TO INTERACT VIA BINDING TO THE ACTIVE SITE OF CYTOCHROME P_1-450

Substrates for enzymes regulated by the Ah gene complex include virtually all important classes of lipophilic environmental chemicals and many common therapeutic drugs.

Although many environmental chemicals are inducers of cytochrome P_1-450 (leading to the variety of responses described above), chemicals which specifically inhibit P_1-450 are far less common. The most studied chemical which inhibits P_1-450 relatively specifically is α-naphthoflavone (ANF; 7,8-benzoflavone). ANF inhibits the ability of 7,12-dimethylbenz(a)anthracene to induce tumors in mouse skin (Gelboin et al. 1970) and reduces the incidence of leukemia induced by benzo(a)pyrene in mice (Nebert 1981). There do not appear to be many environmental chemicals which are potent inhibitors of P_1-450 although certain "weak" carcinogens such as dibenz(a,c)anthracene may reduce the metabolic activation of stronger carcinogens such as 7,12-dimethylbenz(a)anthracene by competition for enzyme active sites (Slaga and Boutwell 1977).

FUTURE DIRECTIONS: USE OF PRINCIPLES DERIVED FROM THE Ah GENE MODEL TO STUDY COMBINED EFFECTS OF XENOBIOTICS

HUMAN Ah PHENOTYPES

There is a need to develop a reliable assay to identify individuals in the human population who are genetically "responsive" vs. "nonresponsive" at the Ah locus. Moreover, we must determine their long-term differential susceptibility to specific xenobiotic exposures.

Does a high degree of "inducibility" result in increased risk (as proposed by Kellermann et al. 1973)?

The most widely used method for human phenotyping, i.e., AHH induction in mitogen-stimulated lymphocytes, is simply too variable to permit evaluation of the role of the Ah locus in human carcinogenesis or toxicity (Atlas and Nebert 1978). Human Ah phenotypes potentially could be determined in vivo by the plasma clearance rates of "probe drugs" whose metabolism is accomplished by the same species of P-450 which metabolize benzo(a)pyrene. Although plasma disappearance rates for antipyrine correlated well with lymphocyte AHH inducibility in a small human population (Kellermann and Luyten-Kellermann 1978), it remains to be demonstrated that antipyrine is predominantly metabolized by enzymes regulated at the Ah locus. Other promising probe drugs, for which ethnic/genetic differences in metabolism exist in man, include debrisoquine (Kalow et al. 1980) and sparteine (Inaba et al. 1980); it is possible that one of these compounds or another drug will be found to be a reliable indicator of individual levels of "responsiveness" at the Ah locus in humans.

Sensitive alternative assays for assessing human Ah phenotypes also might be accomplished by new techniques using radioimmunoassay to detect specific forms of cytochrome P-450 associated with the Ah locus (Negishi and Nebert 1979), or by measurement of Ah receptor levels in lymphocytes which have not been exposed to mitogens. In the near future it also may be possible to measure P_1-450 mRNA levels by RNA-DNA hybridizations using the human P_1-450 cloned gene.

"TOXIFICATION"/"DETOXICATION" BALANCE

There is a need to clarify the balance between toxification (due to metabolic activation) and detoxication (metabolism, conjugation and excretion) related to different levels of induced P_1-450 in liver, bowel and tissues that are portals of entry for xenobiotics. Under what defined circumstances are high levels of induced P_1-450 (due to previous chemical exposure) "protective"? Under what circumstances are high P_1-450 levels deleterious (Nebert 1981)?

MULTIPLE SPECIES OF P-450: REGULATION AND CONSEQUENCES

Can we selectively induce and/or inhibit specific types of P-450 and thereby reduce xenobiotic toxicities? What are the molecular and genetic mechanisms by which multiple forms of cytochrome P-450 are synthesized in response to multiple xenobiotic exposure? Is synthesis of multiple P-450 species controlled by mechanisms analogous to those which regulate secretion of diverse antibody types in response to a wide range of antigens (Nebert et al. 1981)? Are there P-450 species which are primarily "protective" and other P-450 species which are primarily generators of toxic metabolites from xenobiotics?

RECEPTOR ANTAGONISTS

We must develop specific antagonists which can occupy induction-receptor sites, thereby blocking induction of cytochrome P_1-450 and associated enzymes. This would be analogous to the development of "anti-estrogens" which act by competitively inhibiting binding of potent estrogen agonists to the estrogen receptor. Will antagonists to induction of various forms of cytochrome P-450 be capable of altering the toxicity of xenobiotics? At this time there are no chemicals known which are antagonists for binding to the Ah receptor. All compounds which are known to bind to the Ah receptor (assessed by competition with [^{3}H]TCDD) are agonists which induce the cytochrome P_1-450 response.

SPECIFIC RECEPTORS FOR OTHER CLASSES OF XENOBIOTIC INDUCERS

We must attempt to identify new specific molecular sites at which xenobiotics act and interact. We have characterized a receptor associated with the Ah gene complex which regulates induction of cytochrome P_1-450 and associated enzyme activities by "3-methylcholanthrene-type" inducers (including dioxins, PCBs, etc.). Are there also undiscovered specific receptors which mediate induction of other forms of P-450 by such agents as phenobarbital (Poland et al. 1981), etc?

Ah RECEPTOR AS A BIOACTIVITY MONITOR

As described earlier with reference to human Ah phenotypes, competition for Ah receptor binding sites can be used to assess the total biological activity of complex environmental samples. This approach could be applied further as a screening procedure to detect very low levels (activities) of dioxins and dibenzofurans and higher levels of PCBs, PBBs and polycyclic aromatic hydrocarbons. As a screening procedure, this should be considerably more economical than chemical identification of specific isomers present in complex mixtures and should be a convenient index to potential toxicity in such mixtures.

Ah RECEPTOR AS A MEDIATOR OF XENOBIOTIC TOXICITY

With the exception of β-naphthoflavone, virtually all compounds which bind with high affinity to the cytosolic Ah receptor are highly toxic. Indeed, Poland et al. (1979) have proposed that receptor binding per se is a critical step in toxicity caused by chlorinated aromatic hydrocarbons. The generality and validity of this hypothesis deserve further testing which can be accomplished, in part, by exploitation of genetic differences at the Ah locus.

ENDOGENOUS LIGANDS FOR THE Ah RECEPTOR

At present there is no known endogenous (physiologic) compound which binds to the Ah receptor. All chemicals which are known to bind to the receptor with high affinity are of xenobiotic origin. Why should

there exist a receptor which binds foreign chemicals, but has no known physiologic function? Is there an endogenous ligand for the Ah receptor? Does binding of xenobiotic chemicals to this receptor somehow interfere with a critical physiologic function, thereby leading to toxicity?

PHYLOGENY OF RECEPTOR-MEDIATED P_1-450 INDUCTION

Humans are not the only target species for the toxic effects of xenobiotic chemicals. Polycyclic aromatic hydrocarbons are known to induce their own metabolism in mammals, birds, fish and in some invertebrate species. Various forms of cytochrome P-450 are ubiquitously distributed throughout the living world, including bacteria (Nebert et al. 1981). What regulates the activity of P-450-mediated enzymes in all these species? Presently, P_1-450 induction mediated by the Ah receptor in mammals is the only system in which any molecular details are available as to the mechanism of regulation. Is there receptor-mediated regulation of P-450 induction in other species? Is P-450 induction important in the toxic responses of non-mammalian species to xenobiotic chemicals in their environment?

CONCLUSION

The Ah gene complex is a model which has provided considerable information on the mechanism(s) of action of several important xenobiotic chemicals. The model provides specific sites at which xenobiotics may interact and suggests that intervention at particular sites could be effective in reducing the toxicity caused by multiple xenobiotic exposures.

It is clear, however, that these metabolic systems are very complex and that we have barely begun to understand regulation of one single type of P-450. Given the primitive nature of our present understanding, we should consider it possible that existing cytochrome P-450 systems have evolved to a state which represents the optimal balance of P-450 types. We do not yet have the capability to selectively enhance "detoxication" pathways or to selectively suppress "toxification" routes.

Therefore, reduced exposure remains the forced method of choice for reducing the impact of multiple xenobiotic chemicals on man and other species.

REFERENCES

Atlas, S.A. and Nebert, D.W. 1978. Pharmacogenetics: a possible pragmatic perspective in neoplasm predictability. Semin. Oncol. 5: 89-106.

Bandiera, S., Safe, S. and Okey, A.B. 1982. Binding of polychlorinated biphenyls classified as either PB-, MC- or mixed-type inducers to cytosolic Ah receptor. Chem.-Biol. Interact. (in press).

Conney, A.H. 1967. Pharmacological implications of microsomal enzyme induction. Pharmacol. Rev. 19: 317-366.

Conney, A.H., Miller, E.C. and Miller, J.A. 1957. Substrate-induced synthesis and other properties of benz(a)pyrene hydroxylase in rat liver. J. Biol. Chem. 228: 753-766.

Essigmann, J.M., Croy, R.G., Nadzan, A.M., Busby, Jr., W.F., Reinhold, V.N., Buchi, G. and Wogan, G.N. 1977. Structural identification of the major DNA adduct formed by aflatoxin B_1 in vitro. Proc. Natl. Acad. Sci. U.S.A. 74: 1870-1874.

Gelboin, H.V., Wiebel, F.J. and Diamond, L. 1970. Dimethylbenzanthracene tumorigenesis and aryl hydrocarbon hydroxylase in mouse skin. Inhibition by 7,8-benzoflavone. Science 170: 169-171.

Haugen, D.J., Coon, M.J. and Nebert, D.W. 1976. Induction of multiple forms of mouse liver cytochrome P-450. Evidence for genetically controlled de novo protein synthesis in response to treatment with β-naphthoflavone or phenobarbital. J. Biol. Chem. 251: 1817-1827.

Hutzinger, W., Olie, K., Lustenhouwer, J.W.A., Okey, A.B., Bandiera, S. and Safe, S. 1981. Polychlorinated dibenzo-p-dioxins and dibenzofurans: a bioanalytical approach. Chemosphere 10: 19-25.

Inaba, T., Otton, S.V. and Kalow, W. 1980. Deficient metabolism of debrisoquine and sparteine. Clin. Pharmacol. Ther. 27: 547-549.

Kalow, W., Otton, S.V., Kadar, D., Endrenyi, L. and Inaba, T. 1980. Ethnic difference in drug metabolism: debrisoquine 4-hydroxylation in Caucasians and Orientals. Can. J. Physiol. Pharmacol. 58: 1142-1144.

Kellermann, G. and Luyten-Kellermann, M. 1978. Benzo(a)pyrene metabolism and plasma elimination rates of phenacetin, acetanilide and theophylline in man. Pharmacology 17: 191-200.

Kellermann, G., Shaw, C.R. and Luyten-Kellermann, M. 1973. Aryl hydrocarbon hydroxylase inducibility and bronchogenic carcinoma. N. Engl. J. Med. 289: 934-937.

Nebert, D.W. 1979a. Genetic differences in the induction of monooxygenase activities by polycyclic aromatic compounds. Pharmacol. Ther. 6: 395-417.

Nebert, D.W. 1979b. Multiple forms of inducible drug-metabolizing enzymes. A reasonable mechanism by which any organism can cope with adversity. Mol. Cell. Biochem. 27: 27-46.

Nebert, D.W. 1980a. Pharmacogenetics: an approach to understanding chemical and biologic aspects of cancer. J. Natl. Cancer Inst. 64: 1279-1290.

Nebert, D.W. 1980b. The Ah locus: genetic differences in toxic response to foreign compounds. In Microsomes, drug oxidations and chemical carcinogenesis. Vol II. Edited by M.J. Coon, A.H. Conney, R.W. Estabrook, H.V. Gelboin, J.R. Gillette and P.J. O'Brien. Academic Press, N.Y. pp. 801-812

Nebert, D.W. 1981. Genetic differences in suspectibility to chemical induced myelotoxicity and leukemia. Environ. Health Perspect. 39: 11-22.

Nebert, D.W. and Bausserman, L.L. 1970. Genetic differences in the extent of aryl hydrocarbon hydroxylase induction in mouse fetal cell cultures. J. Biol. Chem. 245: 6373-6382.

Nebert, D.W. and Bausserman, L.L. 1971. Aryl hydrocarbon hydroxylase induction in cell culture as a function of gene expression. Ann. N.Y. Acad. Sci. 179: 561-579.

Nebert, D.W. and Jensen, N.M. 1979. The Ah locus: genetic regulation of the metabolism of carcinogens, drugs, and other environmental chemicals by cytochrome P-450-mediated monooxygenases. Edited by G.D. Fasman. CRC Crit. Rev. Biochem. 6(4): 401-437.

Nebert, D.W., Considine, N. and Owens, I.S. 1973. Genetic expression of aryl hydrocarbon hydroxylase induction. VI. Control of other aromatic hydrocarbon-inducible monooxygenase activities at or near the same genetic locus. Arch. Biochem. Biophys. 157: 148-159.

Nebert, D.W., Jensen, N.M., Perry, J.W. and Oka, T. 1980. Association between ornithine decarboxylase induction and the Ah locus in mice treated with polycyclic aromatic compounds. J. Biol. Chem. 255: 6836-6842.

Nebert, D.W., Eisen, H.J., Negishi, M., Lang, M.A., Hjelmeland, L.M. and Okey, A.B. 1981. Genetic mechanisms controlling the induction of polysubstrate monooxygenase (P-450) activities. Annu. Rev. Pharmacol. Toxicol. 21: 431-462.

Negishi, M. and Nebert, D.W. 1979. Structural gene products of the Ah locus. Genetic and immunochemical evidence for two forms of mouse liver cytochrome P-450 induced by 3-methylcholanthrene. J. Biol. Chem. 254: 11015-11023.

Okey, A.B., Bondy, G.P., Mason, M.E., Kahl, G.F., Eisen, H.J., Guenthner, T.M. and Nebert, D.W. 1979. Regulatory gene product of the Ah locus. Characterization of the cytosolic inducer-receptor complex and evidence for its nuclear translocation. J. Biol. Chem. 254: 11636-11648.

Okey, A.B., Bondy, G.P., Mason, M.E., Nebert, D.W., Forster-Gibson, C.J., Muncan, J. and Dufresne, M.J. 1980. Temperature-dependent cytosol-to-nucleus translocation of the Ah receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin in continuous cell culture lines. J. Biol. Chem. 255: 11415-11422.

Poland, A.P., Glover, E., Robinson, J.R. and Nebert, D.W. 1974. Genetic expression of aryl hydrocarbon hydroxylase activity. Induction of monooxygenase activities and cytochrome P_1-450 formation by 2,3,7,8-tetrachlorodibenzo-p-dioxin in mice genetically "nonresponsive" to other aromatic hydrocarbons. J. Biol. Chem. 249: 5599-5606.

Poland, A.P., Glover, E. and Kende, A.S. 1976. Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin by hepatic cytosol. Evidence that the binding species is the receptor for the induction of aryl hydrocarbon hydroxylase. J. Biol. Chem. 251: 4936-4946.

Poland, A., Greenlee, W.F. and Kende, A.S. 1979. Studies on the mechanism of action of the chlorinated dibenzo-p-dioxins and related compounds. Ann. N.Y. Acad. Sci. 320: 214-230.

Poland, A., Mak, E., Glover, E., Boatman, R.J., Ebetino, F.H. and Kende, A.S. 1981. 1,4-Bis(2-[3,5-dichloropyridyloxy])benzene, a potent phenobarbital-like inducer of microsomal monooxygenase activity. Mol. Pharmacol. 18: 571-580.

Remmer, H. and Siegert, M. 1962. Kumulation und elimination von phenobarbital. Arch. Exp. Pathol. Pharmakol. 243: 479-494.

Routledge, P.A. and Shand, D.G. 1979. Presystemic drug elimination. Annu. Rev. Pharmacol. Toxicol. 19: 447-468.

Silinskas, K.C. and Okey, A.B. 1975. Protection by 1,1,1-trichloro-2-2-bis(p-chlorophenyl)ethane (DDT) against mammary tumors and leukemia during prolonged feeding of 7,12-dimethylbenzo(a)-anthracene to female rats. J. Natl. Cancer Inst. 55: 653-657.

Slaga, T.J. and Boutwell, R.K. 1977. Inhibition of the tumor-initiating ability of the potent carcinogen 7,12-dimethylbenz-(a)anthracene by the weak tumor initiator 1,2,3,4-dibenzanthracene. Cancer Res. 37: 128-133.

Wattenberg, L.W. 1978. Inhibition of chemical carcinogensis. J. Natl. Cancer Inst. 60: 11-18.

Weinstein, I.B., Jeffrey, A.M., Jennette, K.W., Blobstein, S.H., Harvey, R.G., Harris, C., Autrup, H., Kasai, H. and Nakanishi, K. 1976. Benzo(a)pyrene diol epoxides as intermediates in nucleic acid binding *in vitro* and *in vivo*. Science 193: 592-595.

METHODOLOGICAL APPROACHES FOR INTERACTION STUDIES: POTENTIATION OF HALOALKANE-INDUCED HEPATOTOXICITY

Gabriel L. Plaa[a] and William R. Hewitt[a]

ABSTRACT

Various methodological approaches have been employed to study a specific form of toxic chemical interaction: the potentiation of haloalkane-induced hepatotoxicity by ketones or ketogenic chemicals. The initial phase concerns itself with the establishment and the characterization of the exposure conditions that provoke the potentiated response. Once these are known, dose-response relationships provide the quantitative information required to determine whether or not the conditions prevailing in the environmental situation under study are favorable for such an interaction. Comparative studies are employed to assess the specificity of the interaction. Finally, mechanistic studies are proposed as the means by which new, more appropriate rapid testing procedures can be expected to be developed.

[a] Département de pharmacologie, Faculté de médecine, Université de Montréal, Montréal, Québec.

DEMARCHES METHODOLOGIQUES POUR DES ETUDES D'INTERACTION: POTENTIALISATION DE L'HEPATOTOXICITE INDUITE PAR LES HALOALCANES

Gabriel L. Plaa[a] et William R. Hewitt[a]

RESUME

Différentes démarches méthodologiques ont été utilisées pour étudier une forme spécifique d'interaction toxique: la potentialisation par les cétones ou les dérivés cétogéniques de l'hépatotoxicité induite par les haloalcanes. La première démarche est effectuée pour caractériser les conditions d'exposition qui provoquent la réponse potentialisée. Dans un deuxième temps, les rapports dose-effet apportent les renseignements quantitatifs nécessaires pour déterminer si les conditions retrouvées dans l'environnement sont favorables à une telle interaction. Des études comparatives sont effectuées pour évaluer la spécificité de l'interaction. Enfin, des études de mécanismes d'action sont proposées dans le but de fournir les éléments nécessaires à la mise au point d'une nouvelle méthodologie plus rapide et plus appropriée.

[a] Département de pharmacologie, Faculté de médecine, Université de Montréal, Montréal, Québec.

INTRODUCTION

The experimental approach to the study of potential toxic interactions between chemical substances is not simple and straightforward. No general protocol can be established at the present time and it is extremely unlikely that one will ever be devised. However, certain approaches have been utilized; these illustrate the problems encountered and possible solutions.

For a number of years the researchers in our laboratory have been interested in chemical interactions that result in potentiation of haloalkane-induced hepatotoxicity and nephrotoxicity. Much of this work deals with poten-tial interactions that might be encountered in an industrial working environment. Individuals exposed to both ethanol and carbon tetra-chloride (CCl_4) are known to be more susceptible to liver injury. Our work started with the well known potentiation of the acute hepatotoxic properties of CCl_4 or chloroform ($CHCl_3$) by ethanol (Kutob and Plaa 1962a; Klaassen and Plaa 1966, 1967). We then progressed to the study of other aliphatic alcohols (Traiger and Plaa 1971) found in the workplace and eventually led to the discovery that certain ketones (acetone, methyl n-butyl ketone, 2,5-hexanedione) or ketogenic chemicals (isopropanol, 1,3-butanediol, n-hexane) could also potentiate haloalkane-induced hepatotoxicity (Traiger and Plaa 1972, 1974; Hewitt and Plaa 1979; Hewitt et al. 1980a). In this regard it is important to note that isopropanol potentiation of CCl_4-induced liver injury, recently described in humans in an occupational setting by Folland et al. (1976), was first described in laboratory animals (Traiger and Plaa 1971, 1972, 1974). In the last 4 years, we have worked with chemical pollutants that are found in water supplies. The possible potentiation of $CHCl_3$-induced liver and kidney injury was the toxic response of interest in these studies. An inte-resting difference in potentiating capacities of two pesticides, chlor-decone and mirex, was observed (Hewitt et al. 1979) and the role of microsomal mixed function oxidases in this potentiation was investigated (Cianflone et al. 1980).

The purpose of our presentation is to discuss the applicability of various approaches to the study of potential toxic interactions. It will be based largely on our own experience in a clearly circumscribed toxic response (liver injury) produced by aliphatic halogenated hydrocarbons. We hope that a wider application to other types of potentially toxic interactions will unfold from the examples that will be described.

CHARACTERIZATION OF CONDITIONS

Before one can conclude whether an interaction may occur under a given set of circumstances, one must first characterize the conditions in which the interaction can be observed. Thus, the experimental conditions are defined in terms of the reproducibility of the interaction, rather than the applicability of the experimental approach to the real-world exposure situation. The question put in its simplest form is:

Can a toxic interaction occur? This means that the dosages selected for experimental purposes have to be in the toxic range. A second characteristic that appears to be essential is that the toxic response under study be dose-dependent: as one increases or decreases the dose administered, the toxic effect varies accordingly. Numerous reports in the literature demonstrate that with CCl_4 and $CHCl_3$, the acute hepatotoxic response is dose-dependent and highly reproducible, particularly in rats or mice, and that it can be reliably measured by biochemical procedures. In all of our studies, we have selected as the challenge dose that amount of CCl_4 or $CHCl_3$ which will cause only a slight degree of liver injury in vehicle-pretreated (control) animals. The possible potentiation is then reflected as an exaggerated response to this challenge dose in animals pretreated with the chemical under study.

Table 1 summarizes the potentiations observed in mice pretreated with various aliphatic alcohols and then subjected to a challenge dose of CCl_4. A similar summary for the potentiation of $CHCl_3$-induced liver injury by various halocarbons is found in Table 2. Hepatotoxicity was assessed by elevations in plasma transaminase activity and/or ornithine carbamyl transferase activity; liver sections were also examined by light microscopy to confirm the biochemical findings. It is evident that by this approach one can find chemicals that can potentiate CCl_4 or $CHCl_3$ liver injury, but that others are devoid of such action.

CHARACTERISTICS OF THE DOSE-RESPONSE RELATIONSHIPS

Haloalkane Component

Theoretically, potentiation of CCl_4 or $CHCl_3$ liver injury could manifest itself in different ways: either the pretreatment affects all animals in such a way that lower dosages of the haloalkane will provoke the hepatotoxic response (a normally noneffective dosage of CCl_4 or $CHCl_3$ will become an effective dosage in pretreated animals) or the effective dosage has not been altered, but the hepatotoxic response to this dosage is exaggerated. In the first situation the dose-response curve has shifted to the left, but remains parallel. In the second, the curve does not shift laterally, but the slope of the dose-response curve becomes steeper. A third alternative exists where both lower dosages are found to provoke a response and the response itself is exaggerated; the dose-response curve shifts laterally, and the slope becomes steeper. These three hypothetical possibilities are depicted in Figure 1. Of course, more complex dose-response relationships are feasible (biphasic, curvilinear, etc). Such cases must be considered on an individual basis.

The implications associated with each situation are different. When the dose-response curve has shifted, but remains parallel, the minimally effective dosage is smaller. However, one can estimate it from the median effective dosage (ED50) since the lines are parallel. Therefore, one can estimate with relative accuracy the dosage threshold required for injury. In the real-world situation, however, one can no

Table 1. Effect of the single administration of aliphatic alcohols on carbon tetrachloride-induced hepatotoxicity in male mice[α] (data from Traiger and Plaa 1971).

Pretreatment	GPT Activity
Vehicle	43 - 55[β]
Methanol (10 mL/kg)	296 ± 24[γ]
n-Propyl alcohol (1.75 mL/kg)	63 ± 9
Isopropanol (2.5 mL/kg)	2147 ± 790[γ]
n-Butyl alcohol (3 mL/kg)	50 ± 5
4-Methyl-2-pentanol (1.4 mL/kg)	77 ± 11

[α] Mice were given CCl_4 (0.0075 mL/kg, ip) 18 h following pretreatment with vehicle or test agent. The animals were sacrificed 24 h later. Values are means ± SE. GPT = glutamic-pyruvic transaminase (units/mL).

[β] Vehicle-pretreated groups were included in each experiment. Values are range of means obtained in 5 experiments.

[γ] Values significantly different from corresponding vehicle-pretreated group ($p < 0.05$).

Table 2. Effect of the single administration of various halocarbons on chloroform-induced hepatotoxicity in male mice[α].

Pretreatment	GPT Activity	OCT Activity
Vehicle	141 - 174[β]	34 - 57[β]
Chlordecone (50 mg/kg)	5027 ± 901[γ]	1916 ± 246[γ]
Mirex (250 mg/kg)	228 ± 13	70 ± 15
Photomirex (100 mg/kg)	145 ± 5	61 ± 16
Hexachlorobenzene (500 mg/kg)	140 ± 26	---
Firemaster BP-6 (500 mg/kg)	185 ± 56	27 ± 3
Aroclor 1254 (500 mg/kg)	264 ± 48[γ]	31 ± 6
Bromodichloromethane (250 mg/kg)	125 ± 21	33 ± 13
1,2,4,5-Tetrachlorobenzene (500 mg/kg)	206 ± 38	34 ± 7
Hexachloroethane (500 mg/kg)	141 ± 18	30 ± 5
9,10-Dichlorostearic acid (1000 mg/kg)	172 ± 21	29 ± 5

α Mice were given $CHCl_3$ (0.1 mL/kg, po) 18 h following pretreatment with vehicle or test agent. The animals were sacrificed 24 h later. Values are means ± SE (standard error). OCT = ornithine carbamyl transferase (nmoles converted/mL).

β Vehicle-pretreated groups were included in each experiment. Values are range of means obtained in 5 experiments.

γ Values significantly different from corresponding vehicle-pretreated group ($p < 0.05$).

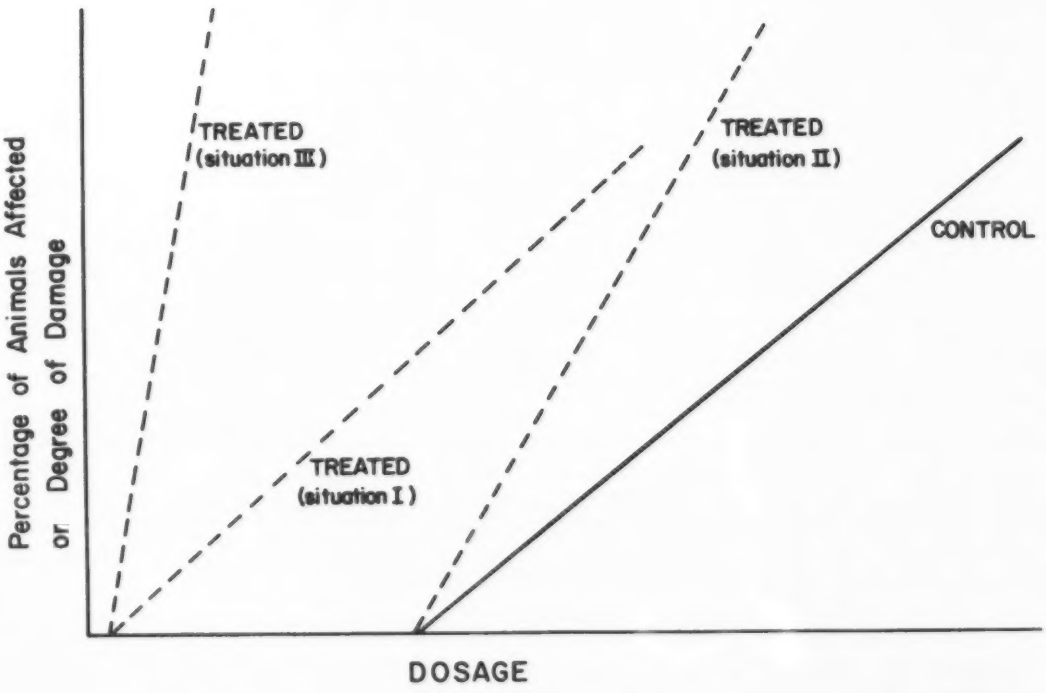


Figure 1. Theoretical shifts in hepatotoxic dose-response curves . following pretreatment with potentiating agents. For convenience these curves are drawn as linear functions. It is assumed that "effect" and "dosage" have been transformed to yield a linear relationship.

longer rely on the "no effect level" established in the absence of the interacting chemical. When the dose-response line has rotated, but has not shifted laterally, the "no effect level" established in the absence of the interacting chemical is still operative when the second chemical is present. However, one cannot predict the severity of the toxic response. In the third situation, nothing can be predicted since both the "no effect level" and the response have changed.

Unfortunately, there are few data available to determine which situation prevails in the interactions that we have studied. The data in Tables 3 and 4 depict the effect of chlordecone on the $CHCl_3$ dose-response relationships (degree of damage and frequency) for hepatotoxicity in mice. Figure 2 is the graphic representation of the GPT activity dose-response data (log transformations) contained in Table 3; GPT activity is an index of degree of damage. Chlordecone treatment results in a lateral shift of the $CHCl_3$ dose-response curve (Situation I, Figure 1). When the percentage of mice exhibiting hepatotoxicity (Table 3) was plotted, the same lateral shift was observed (Figure 3). Median effective dosages (ED50) for production of hepatotoxicity by $CHCl_3$ were calculated from these rats (probit-log dose transformations). These are given in Table 5. Analysis of the transformed dose-response curves indicated that they were parallel to each other. Again, the data show that chlordecone treatment results in a lateral shift of the $CHCl_3$ frequency dose-response curve in mice.

Preliminary data are available for the same interactions in rats (Table 6). Graphical representation (Figure 4) of the GPT activity dose-response data (log transformations) suggests that chlordecone pretreatment results in a lateral shift and in a rotation of the $CHCl_3$ dose-response curve (Situation III, Figure 1) in rats. Further experiments are needed to clarify this point.

In Table 6, data for mirex have been included. This nonketonic analog of chlordecone does not potentiate $CHCl_3$ hepatotoxicity in mice (Hewitt et al. 1979). However, mirex does exert a moderate potentiation in rats. From the preliminary data in Table 6, mirex pretreatment appears to result in a rotation, but not in a lateral shift, of the $CHCl_3$ dose-response curve (Situation II, Figure 1) in rats. This requires clarification.

Pretreatment Component

Up to now, the discussion has dealt with the dose-response relationships associated with the active toxicant of the interacting pair of chemicals, that is, the haloalkane which produces the hepatotoxic response. In order to continue the characterization of the interaction one has to establish the dose-response relationships associated with the pretreating agent that leads to potentiation. In this situation the dosages of the agent are varied and a fixed challenge dose of the haloalkane is administered. A clear dose-response relationship has been observed with 1,3-butanediol potentiation of CCl_4 (Hewitt et al. 1980b).

Table 3. Effect of chlordecone pretreatment on the chloroform plasma GPT dose-response relationship in male mice[α].

$CHCl_3$ (μL/kg)	Vehicle-Pretreated GPT Activity[β]	Vehicle-Pretreated % Abnormal[γ]	Chlordecone-Pretreated GPT Activity[β]	Chlordecone-Pretreated % Abnormal[γ]
2.5	36	11	36	0
5	30	6	44	0
10	27	0	53	36
15	34	0	65	67
25	39	11	242	85
35	58	33	---	---
50	70	56	744	100
75	115	92	1803	100
100	155	100	4307	100
250	824	100		
500	3788	100		
750	4616	100		
1000	5891	100		

[α] Mice were given $CHCl_3$ (po) 18 h following pretreatment with vehicle or chlordecone (50 mg/kg, po). The animals were sacrificed 24 h later.

[β] Values are plasma means (units/mL) for each group.

[γ] Values are percentage of animals in group exhibiting an abnormal plasma GPT value (greater than $\bar{X}$ (mean) + 2 SD (standard deviation) for normal mice not treated with $CHCl_3$).

Table 4. Effect of chlordecone pretreatment on the chloroform plasma OCT dose-response relationship in male mice[α].

$CHCl_3$ (μL/kg)	Vehicle-Pretreated OCT Activity[β]	% Abnormal[γ]	Chlordecone-Pretreated OCT Activity[β]	% Abnormal
2.5	56	5	40	0
5	56	6	70	9
10	101	6	113	20
15	13	0	227	50
25	51	5	193	23
35	21	0	---	---
50	53	6	292	71
75	66	8	364	67
100	108	14	811	100
250	273	44		
500	774	81		
750	735	94		
1000	801	100		

α Mice were given $CHCl_3$ (po) 18 h following pretreatment with vehicle or chlordecone (50 mg/kg, po). The animals were sacrificed 24 h later.

β Values are plasma means (nmoles converted/mL) for each group.

γ Values are percentage of animals in group exhibiting an abnormal plasma OCT value (greater than $\bar{X}$ (mean) + 2 SD (standard deviation) for normal mice not treated with $CHCl_3$).

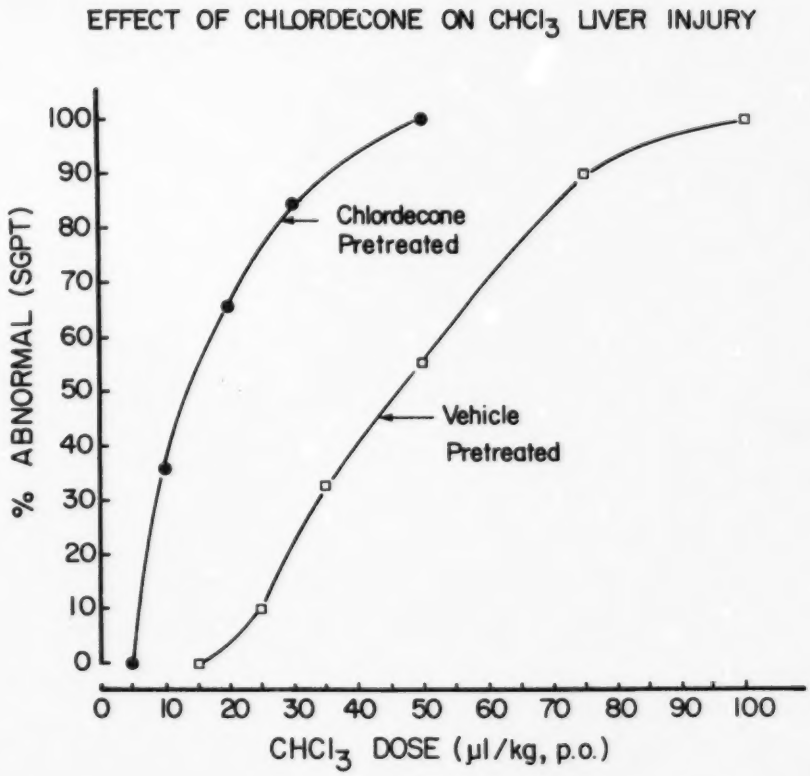


Figure 2. Effect of chlordecone on the chloroform GPT activity dose-response curve in mice. The data appear in Table 3.

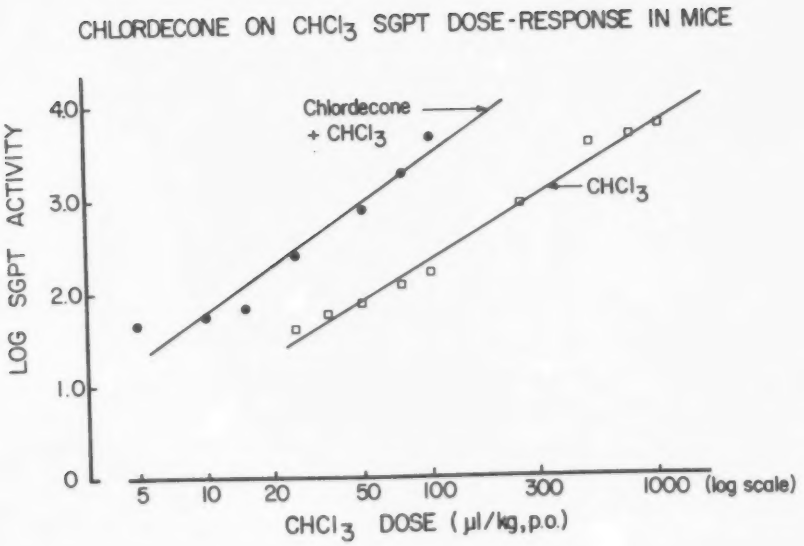


Figure 3. Effect of chlordecone on the incidence of chloroform-induced heptatotoxicity in mice. The data appear in Table 3.

Table 5. Median effective dosages (ED50) for chloroform-induced hepatotoxicity in male mice.[α]

Pretreatment	GPT-ED50 (μL/kg)	Potency Ratio[β]	OCT-ED50 (μL/kg)	Potency Ratio
Vehicle	43 (35-50)[γ]	---	235 (184-301)	---
Chlordecone	13 (10-17)	3.2 (1.3-7.8)	28 (20-43)	8.3 (5.3-12.8)

[α] Data for calculations obtained from Tables 3 and 4.

[β] Potency Ratio = Vehicle ED50/Chlordecone ED50.

[γ] Values in parentheses represent the 95% confidence limits.

Table 6. Effect of chlordecone and mirex pretreatment on the chloroform plasma GPT and OCT dose-response relationship in male rats[α].

$CHCl_3$ (μL/kg)	Vehicle-Treated	Chlordecone-Treated	Vehicle-Treated	Mirex-Treated
		GPT Activity		
50	43 ± 3	111 ± 40	41 ± 2	64 ± 8
75	43 ± 3	210 ± 54	53 ± 5	68 ± 4
100	59 ± 10	941 ± 474	59 ± 14	74 ± 11
250	94 ± 39	2978 ± 375	133 ± 44	157 ± 29
500	163 ± 22	2793 ± 482	146 ± 59	469 ± 88
		OCT Activity		
50	2 ± 0.3	38 ± 26	3 ± 0.9	6 ± 2
75	3 ± 0.4	120 ± 41	3 ± 0.5	6 ± 2
100	9 ± 5	556 ± 206	3 ± 0.5	10 ± 4
250	39 ± 29	1736 ± 512	91 ± 49	42 ± 12
500	72 ± 19	1548 ± 83	63 ± 22	197 ± 90

[α] Rats were given $CHCl_3$ (po) 18 h following pretreatment with vehicle, chlordecone (50 mg/kg, po) or mirex (50 mg/kg, po). The animals were sacrificed 24 h later. Values are means ± SE.

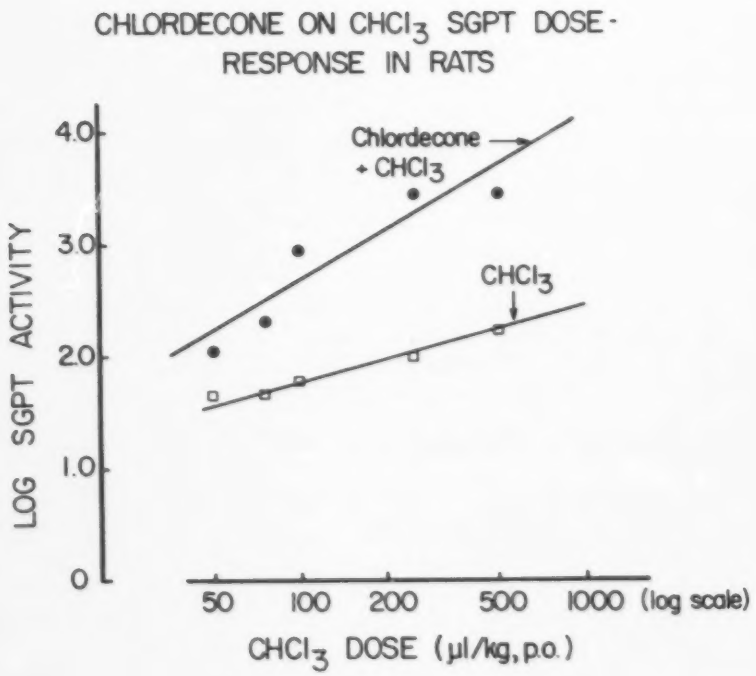


Figure 4. Effect of chlordecone on the chloroform GPT activity dose-response curve in rats. The data appear in Table 6.

With the isopropanol-CCl_4 and the acetone-CCl_4 models, these characteristics have been described (Plaa and Traiger 1973; Plaa et al. 1982) and have led to important conclusions. Figure 5 depicts the dose-response curves for both isopropanol and acetone. From these data we concluded that in the isopropanol potentiation of CCl_4 hepatotoxicity, acetone, the ketonic metabolite of isopropanol, was largely responsible for the potentiation. This hypothesis was substantiated by other experiments (Traiger and Plaa 1972). The observation led to the development of a more general hypothesis that ketonic solvents or ketogenic solvents could be suspected as possible potentiators of haloalkane-induced hepatotoxicity (Hewitt et al. 1980b).

Using the acetone-CCl_4 model we further demonstrated that a minimally effective dosage (MED) and a noneffective dosage (NED) could be established in laboratory animals (Table 7). The fact that such dosages could be established permitted us to demonstrate that a threshold blood concentration of acetone does exist, below which potentiation of CCl_4 does not occur (Plaa et al. 1982). The implications of these findings to the prevention of such an interaction in the workplace are of considerable importance, since they should assist in the establishment of safer occupational environments.

With chlordecone potentiation of $CHCl_3$ hepatotoxicity, only preliminary data are available. However, in mice a clear dose-response relationship has been observed (Table 8). Current studies indicate that the same relationship can be demonstrated in rats.

DOSAGES AND THE ENVIRONMENTAL SITUATION

The experimental design of dose-response experiments is largely dependent upon the actual exposure conditions one might expect to occur in the real-world situation. The examples that have been given are appropriate when one is anticipating single exposure conditions, like an accidental spill or a very abrupt short-term exposure. However, when the exposures are likely to be more prolonged, such designs have very limited applicability. Nevertheless, they do assist in the establishment of more appropriate experimental designs.

An example of a more difficult design is one that we encountered for interaction studies with chlordecone or mirex in combination with $CHCl_3$. Initially we established the water concentrations of each pesticide to which the rats would have to be exposed via the drinking water to potentiate a challenge dose of $CHCl_3$. These data are summarized in Table 9. Although the variability was high, the results show that with this design chlordecone and mirex potentiated $CHCl_3$ liver injury at exposure concentrations of 10 ppm. Subsequently, we exposed rats to these concentrations of the two pesticides for 4 weeks. However, after 2 weeks $CHCl_3$ was also added at varying concentrations to the drinking water (Table 10). The data show that under these exposure conditions potentiation of hepatotoxicity was not observed. This type of design approximates more closely the conditions

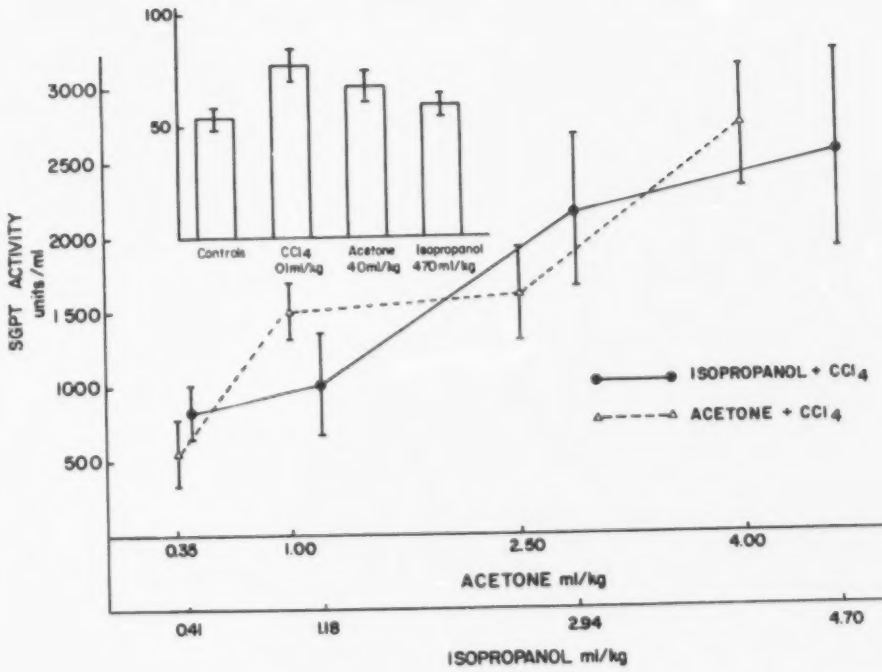


Figure 5. Dose-response curves for isopropanol and acetone for potentiation of carbon tetrachloride-induced hepatotoxicity in male rats (data from Plaa and Traiger 1973).

Table 7. Dose-response relationships for isopropanol and acetone on carbon tetrachloride hepatotoxicity in male rats[a] (data from Plaa et al. (1982).

Dosage (mL/kg)	GPT Activity	OCT Activity
	Isopropanol	
0.025	36 ± 6	9 ± 5
0.050	34 ± 3	6 ± 2
0.10	34 ± 1	18 ± 11
0.25	79 ± 16	35 ± 9
0.50	260 ± 61	453 ± 206
1.00	831 ± 213	961 ± 296
2.50	1220 ± 245	1369 ± 149
	Acetone	
0.025	38 ± 5	8 ± 3
0.050	39 ± 4	12 ± 6
0.10	85 ± 23	13 ± 4
0.25	213 ± 104	158 ± 131
0.50	539 ± 161	246 ± 118
1.00	1777 ± 542	1201 ± 347
2.50	1520 ± 522	1047 ± 214

Noneffective dosage: ~ 0.10 mL/kg
Minimally effective dosage: ~ 0.25 mL/kg

[a] CCl_4 (0.1 mL/kg, ip) was administered 18 h following pretreatment with isopropanol or acetone. The animals were sacrificed 24 h later. Values are means ± SE.

Table 8. Dose-response relationships for chlordecone on chloroform hepatotoxicity in male mice[a].

Dosage (mg/kg)	GPT Activity	OCT Activity
0	143 ± 9	214 ± 51
1	152 ± 9	85 ± 13
2.5	166 ± 36	151 ± 126
5	292 ± 72	126 ± 33
10	740 ± 171	256 ± 52
25	1600 ± 299	701 ± 128
50	3057 ± 509	879 ± 144

[a] $CHCl_3$ (0.1 mL/kg, po) was administered 18 h following pretreatment with vehicle or chlordecone. The animals were sacrificed 24 h later. Values are means ± SE.

Table 9. Effect of chlordecone or mirex pretreatment (2 and 4 weeks) on chloroform hepatotoxicity in male rats[α].

Pretreatment	Glutamic-pyruvic Transamine Activity Two weeks	Four Weeks
Vehicle	169 ± 18	148 ± 0
Chlordecone (0.1 ppm)	249 ± 120	238 ± 74
Chlordecone (1.0 ppm)	288 ± 187	355 ± 65
Chlordecone (10.0 ppm)	2178 ± 866	3750 ± 2490
Vehicle	428 ± 107	266 ± 144
Mirex (0.1 ppm)	371 ± 155	124 ± 10
Mirex (1.0 ppm)	257 ± 71	268 ± 102
Mirex (10.0 ppm)	1289 ± 1170	440 ± 30

[α] Rats were maintained on water containing 0.5% Tween 80 (vehicle) or vehicle + test agent for 2 or 4 weeks. On Day 15 or Day 29, the rats were challenged with $CHCl_3$ (0.5 mL/kg, po). The animals were killed 24 h after $CHCl_3$ administration. Values are means ± SE.

Table 10. Hepatotoxic effects of drinking water containing chlordecone or mirex (10 ppm) and varying concentrations of chloroform in male rats[a].

Water Composition		
Pretreatment Period	Challenge Period	GPT Activity
Vehicle	$CHCl_3$ (50 ppb)	42 ± 4
Vehicle	$CHCl_3$ (5 ppm)	38 ± 1
Vehicle	$CHCl_3$ (500 ppm)	67 ± 9
Chlordecone	Chlordecone + $CHCl_3$ (50 ppb)	50 ± 7
Chlordecone	Chlordecone + $CHCl_3$ (5 ppm)	46 ± 5
Chlordecone	Chlordecone + $CHCl_3$ (500 ppm)	48 ± 2
Vehicle	$CHCl_3$ (50 ppb)	29 ± 3
Vehicle	$CHCl_3$ (5 ppm)	30 ± 4
Vehicle	$CHCl_3$ (500 ppm)	36 ± 2
Mirex	Mirex + $CHCl_3$ (50 ppb)	47 ± 6
Mirex	Mirex + $CHCl_3$ (5 ppm)	38 ± 7
Mirex	Mirex + $CHCl_3$ (500 ppm)	36 ± 8

[a] Rats were maintained on water containing 0.5% Tween 80 (vehicle) or vehicle + test agent for 2 weeks (Pretreatment Period). At the end of this period (Day 15), $CHCl_3$ was introduced into the drinking water; the animals were maintained on these solutions for an additional 2 weeks (Challenge Period). The animals were killed on Day 29. Values are means ± SE.

one might find in a contaminated environment. Of course when long-term exposures to such combinations are of concern the experimental design must be modified accordingly.

SPECIFICITY OF THE INTERACTION

POTENTIATION OF CHLORINATED METHANES AND ETHANES

The interactions that have been described have largely been effected with CCl_4 and $CHCl_3$. An obvious question is whether such interactions can also be observed with other haloalkanes. It is well established that hepatotoxic potency varies amongst halogenated methanes and ethanes (Plaa et al. 1958; Kutob and Plaa 1962b; Klaassen and Plaa 1966, 1967). With the weaker hepatotoxic haloalkanes it is important to determine if their potency is increased as the result of such interactions. With ethanol, isopropanol or acetone the weak hepatotoxicants are either only slightly potentiated (trichloroethylene, 1,1,2-trichloroethane) or are unaffected (1,1,1-trichloroethane) (Klaassen and Plaa 1966, 1967; Traiger and Plaa 1974). Consequently, with these potentiators and these haloalkanes, a weak hepatotoxicant is not converted into a strong hepatotoxicant (potentiated response is not like that observed with CCl_4 or $CHCl_3$).

POTENTIATION OF BROMINATED METHANES

Only preliminary data have been accumulated with chlordecone as the potentiating agent. When chlordecone was tested against brominated methanes (tribromomethane, dibromochloromethane, bromodichloromethane), the picture was less reassuring. These three derivatives can be considered as weak hepatotoxicants since they fail to produce marked hepatoxicity when administered at near-lethal dosages. When rats were subjected to chlordecone pretreatment and then challenged with these brominated methanes, a striking potentiation was observed with dibromochloromethane and bromodichloromethane, but not with tribromomethane (Table 11). The disturbing feature is that although large dosages (relative to lethality) of the compounds are still required for production of liver injury, the hepatotoxic response is greatly exaggerated in the potentiated state. This suggests that Situation III depicted in Figure 1 may prevail with dibromochloromethane. Preliminary results indicate that a similar pattern may exist when acetone is employed as the pretreating agent. Thus, it is now clear that generalizations regarding conversion of "weak hepatotoxicants" to "strong hepatotoxicants" cannot be made at this time; predictions regarding outcomes of such interactions must be guarded and preferably restricted to specific interacting pairs of agents.

Table 11. Effect of chlordecone pretreatment on the hepatotoxicity of brominated methane derivatives in male rats.

Haloalkane challenge	Vehicle	GPT Activity after Pretreatment Chlordecone[α]	Chlordecone (3X)[β]
Tribromomethane			
0.1 mL/kg	41 ± 2	70 ± 13	52 ± 5
0.5 mL/kg	45 ± 6	66 ± 7	400 ± 128
Dibromochloromethane			
0.1 mL/kg	47 ± 4	347 ± 119	103 ± 25
0.5 mL/kg	42 ± 9	888 ± 125	2500 ± 1421
Bromodichloromethane			
0.1 mL/kg	42 ± 3	728 ± 187	1132 ± 556
0.5 mL/kg	76 ± 18	2393 ± 610	3439 ± 953

[α] Rats were pretreated with vehicle or chlordecone (50 mg/kg, po) 18 h before receiving the oral challenge dose of the brominated methane derivative. The animals were killed 24 h after administration of the haloalkane. Values are means ± SE.

[β] Rats were pretreated with vehicle or chlordecone (10 mg/kg/d, po, for 3 d) before receiving the oral challenge dose of the brominated methane derivative. The animals were killed 24 h after the administration of the haloalkane. Values were means ± SE.

DEVELOPMENT OF RAPID IDENTIFICATION TESTS

MIXED FUNCTION OXIDASE ACTIVITY

In order to deal with multiple interacting combinations that one might face, there is a real need for rapid tests that can be used to identify which combinations are most likely to require extensive testing. The mechanisms of action involved in such interactions might furnish such rapid testing procedures. We have approached this aspect of the problem with interactions leading to potentiation of haloalkane-induced hepatotoxicity. With CCl_4 and $CHCl_3$ other investigators have established that bioactivation of these haloalkanes to toxic reactive metabolites is an obligatory phase preceding the appearance of the hepatic lesion. Furthermore, the bioactivation occurs through the hepatic MFO system. Therefore, one could expect that measurement of MFO activity and cytochrome P-450 content following treatment with the test agents might indirectly identify potential interacting agents. However, the data in Table 12 indicate that in mice pretreated with chlordecone and mirex, there was a very poor correlation between increased MFO activity or cytochrome P-450 content and the potentiating properties (Table 2) of these pesticides. Mirex, which failed to potentiate $CHCl_3$, increased MFO activity and increased cytochrome P-450 content; the potentiator chlordecone exerted no effect on cytochrome P-450 content and had only a partial effect on MFO activity. These results indicate that predictions based only on these two parameters would have been erroneous. Consequently, MFO activity or cytochrome P-450 content should be used with caution.

IRREVERSIBLE BINDING TO MACROMOLECULES

A more adequate means of assessing bioactivation of $CHCl_3$ or CCl_4 is to measure the irreversible binding of the metabolites to hepatic macromolecules, with use of radiolabeled substrates. We performed such experiments in mice pretreated with chlordecone and mirex. Table 13 reveals that both *in vivo* and *in vitro* chlordecone treatment resulted in enhanced irreversible binding of $^{14}CHCl_3$ whereas mirex failed to influence binding. Thus, a correlation existed between binding to macromolecules and potentiation of hepatotoxicity. The preliminary data suggest that such procedures might be adaptable to the development of a rapid testing system.

ROLE OF MECHANISTIC STUDIES

For interaction studies, it is obvious that the number of chemical combinations that can be thoroughly investigated in the laboratory is extremely small relative to the great number of chemical combinations that exist in the environment. Therefore, there is an urgent need to develop simpler testing systems with reasonable predictive qualities. Different testing systems are needed for different types of

Table 12. Effects of chlordecone and mirex on mixed function ox. activity in male mice[α] (data from Cianflone et al. 1980).

Treatment	Aminopyrine N-demethylase activity (nmol/mg protein/min)	Aniline Hydroxylase Activity (nmol/mg protein/min)	Cytochrome P-450 Content (nmol/mg protein)
Vehicle	19.9 ± 1.1	2.12 ± 0.08	0.71 ± 0.01
Chlordecone	25.9 ± 1.8[β]	2.38 ± 0.08	0.78 ± 0.07
Mirex	37.1 ± 1.1[β,γ]	2.78 ± 0.08[β,γ]	0.95 ± 0.07[β,γ]

[α] Mice received vehicle, chlordecone (50 mg/kg, po) or mirex (50 mg/kg, po). The animals were sacrificed 18 h later and their livers processed for measurement of microsomal activity. Values are means ± SE.

[β] Significantly different from vehicle-treated group ($p < 0.05$).

[γ] Significantly different from chlordecone-treated group ($p < 0.05$).

Table 13. Effects of chlordecone and mirex on covalent binding of [^{14}C]-chloroform to liver constituents in male mice[α] (data from Cianflone et al. 1980).

Treatment	Binding to Protein[β]	Binding to Lipid[γ]
	In Vivo	
Vehicle	0.62 ± 0.15	0.50 ± 0.05
Chlordecone	2.41 ± 0.31[δ]	1.66 ± 0.16[δ]
Mirex	0.61 ± 0.09	0.43 ± 0.03
	In Vitro	
Vehicle	129 ± 4	---
Chlordecone	188 ± 21[δ]	---
Mirex	114 ± 2	---

[α] In Vivo Experiment: Mice were challenged with $^{14}CHCl_3$ (0.1 mL/kg, po) 18 h after administration of vehicle, chlordecone (50 mg/kg, po) or mirex (50 mg/kg, po). The animals were sacrificed 3 h after $^{14}CHCl_3$ administration. In Vitro Experiment: Mice received vehicle, chlordecone or mirex. The animals were sacrificed 18 h later. Microsomes were prepared and incubated with $^{14}CHCl_3$. Values are means ± SE.

[β] Units for in vivo experiment: nmol/mg protein. Units for in vitro experiment: pmol/mg protein/min.

[γ] Units: nmol/mg lipid.

[δ] Significantly different from vehicle-treated group ($p < 0.05$).

biological effects. Mechanistic studies that identify the various stages involved in the evolution of the biologic response can be particularly rewarding in this regard. However, most investigators who are actively engaged in such studies are rarely the ones who are also concerned about the potential effects of chemical combinations. Therefore, it appears essential that those of us who do investigate potential toxic chemical interactions should look closely at ongoing fundamental mechanistic research in a different light. We should attempt to uncover those key concepts about mechanisms of action that can be applied to the development of test systems for the situations of concern.

In this manner, the eventual complete biological assessment of potentially hazardous chemical combinations should become more rational and less empirical.

ACKNOWLEDGMENTS

We gratefully acknowledge the expert technical assistance of Johanne Couture and Monique Morisset. Financial support for the previously unpublished data presented herein was provided by contracts OSU77-0010, OSU78-00131 and OST79-00014 from The Environmental Health Directorate, Health and Welfare Canada and a Strategic Grant from NSERC.

REFERENCES

Cianflone, D.J., Hewitt, W.R., Villeneuve, D.C. and Plaa, G.L. 1980. Role of biotransformation in the alterations of chloroform hepatotoxicity produced by Kepone and mirex. Toxicol. Appl. Pharmacol. 53: 140-149.

Folland, D.S., Schaffner, W., Grinn, H.E., Crofford, Q.B. and McMurry, D.R. 1976. Carbon tetrachloride toxicity potentiated by isopropyl alcohol. Investigation of an industrial outbreak. J. Am. Med. Assoc. 236: 1853-1856.

Hewitt, W.R. and Plaa, G.L. 1979. Potentiation of carbon tetrachloride-induced hepatotoxicity by 1,3-butanediol. Toxicol. Appl. Pharmacol. 47: 177-180.

Hewitt, W.R., Miyajima, H., Côté, M.G. and Plaa, G.L. 1979. Acute alteration of chloroform-induced hepato- and nephrotoxicity by mirex and Kepone. Toxicol. Appl. Pharmacol. 48: 509-527.

Hewitt, W.R., Miyajima, H., Côté, M.G. and Plaa, G.L. 1980a. Acute alteration of chloroform-induced hepato- and nephrotoxicity by n-hexane, methyl n-butyl ketone, and 2,5-hexanedione. Toxicol. Appl. Pharmacol. 53: 230-248.

Hewitt, W.R., Miyajima, H., Côté, M.G. and Plaa, G.L. 1980b. Modification of haloalkane-induced hepatotoxicity by exogenous ketones and metabolic ketosis. Fed. Proc. 39: 3118-3123.

Klaassen, C.D. and Plaa, G.L. 1966. Relative effects of various chlorinated hydrocarbons on liver and kidney function in mice. Toxicol. Appl. Pharmacol. 9: 139-151.

Klaassen, C.D. and Plaa, G.L. 1967. Relative effects of various chlorinated hydrocarbons on liver and kidney function in dogs. Toxicol. Appl. Pharmacol. 10: 119-131.

Kutob, S.D. and Plaa, G.L. 1962a. The effect of acute ethanol intoxication on chloroform-induced liver damage. J. Pharmacol. Exp. Ther. 96: 99-113.

Kutob, S.D. and Plaa, G.L. 1962b. A procedure for estimating the hepatotoxic potential of certain industrial solvents. Toxicol. Appl. Pharmacol. 4: 354-361.

Plaa, G.L. and Traiger, G.J. 1973. Mechanism of potentiation of CCl_4-induced hepatotoxicity. In Pharmacology and the future of man, Vol. 2. Karger, Basle. pp. 100-113.

Plaa, G.L., Evans, E.A. and Hine, C.H. 1958. Relative hepatotoxicity of seven halogenated hydrocarbons. J. Pharmacol. Exp. Ther. 123: 224-229.

Plaa, G.L., Hewitt, W.R., du Souich, P., Caillé, G. and Lock, S. 1982. Isopropanol and acetone potentiation of carbon tetrachloride-induced hepatotoxicity: Single versus repetitive pretreatments in rats. J. Toxicol. Environ. Health (in press).

Traiger, G.J. and Plaa, G.L. 1971. Differences in the potentiation of carbon tetrachloride in rats by ethanol and isopropanol pretreatment. Toxicol. Appl. Pharmacol. 20: 105-112.

Traiger, G.J. and Plaa, G.L. 1972. Relationship of alcohol metabolism to the potentiation of CCl_4 hepatotoxicity induced by aliphatic alcohols. J. Pharmacol. Exp. Ther. 183: 481-488.

Traiger, G.J. and Plaa, G.L. 1974. Chlorinated hydrocarbon toxicity. Potentiation by isopropyl alcohol and acetone. Arch. Environ. Health 28: 276-278.

ENDOGENOUS MECHANISMS FOR THE REGULATION OF HEPATIC MICROSOMAL DRUG AND STEROID MIXED FUNCTION OXIDASES

G.D. Bellward, R.C.K. Pak, L.S. Gontovnick, B.L. Warren, M.J.P. Finlayson and G.I. Sunahara

ABSTRACT

Various cytosolic or membrane bound receptors for hormones have been found to exist in the liver, e.g. binding sites for androgens, estrogens, prolactin and growth hormone. There is suggestive evidence that changes in number or affinity of certain of these receptors can be correlated with changes in the mixed function oxidase (MFO) enzymes responsible for metabolism of endogenous and exogenous compounds. It is known that manipulation of hormone levels or treatment with various chemicals can produce changes, in some instances permanent changes, in the activity of these MFO enzyme systems. Since there is a highly complex interplay of hormonal control mechanisms in the hypothalamic-pituitary-end organ system, disruption of this system at any point in the cycle, including the MFO enzymes, may result in significant toxic effects. Therefore, it is important to clarify what the interplay is between these enzymes and generalized hormone-mediated cellular responses. In order to achieve this goal, researchers in the area will have to take a much broader "holistic" approach than has generally been the case.

Division of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia.

MECANISMES ENDOGENES ASSURANT LA REGULATION DES OXYDASES A FONCTIONS MULTIPLES DES MICROSOMES HEPATIQUES RESPONSABLES DE LA DEGRADATION DES MEDICAMENTS ET DES STEROIDES

G.D. Bellward, R.C.K. Pak, L.S. Gontovnick, B.L. Warren, M.J.P. Finlayson et G.I. Sunahara

RESUME

Il existe dans le foie divers récepteurs cytosoliques ou liés à une membrane qui sont sensibles aux hormones, p. ex. sites de liaison des androgènes, des prolactines et des hormones de croissance. Selon certaines données, des modifications au niveau du nombre ou de l'affinité de ces récepteurs se traduiraient par des changements au niveau des oxydases à fonctions multiples responsables du métabolisme de composés endogènes et exogènes. Des variations des taux d'hormone ou l'application de traitements comportant divers produits chimiques peuvent modifier, et parfois de façon permanente, l'activité de ces systèmes enzymatiques. Vu l'interaction très complexe des mécanismes intervenant lors du contrôle hormonal dans le système hypothalamus-hypophyse-organe contrôlé, il se peut que le fait de perturber ce système à l'un quelconque des points du cycle, y compris au niveau des oxydases à fonctions multiples, se traduise par d'importants effets toxiques. Il est donc important d'élucider la nature de ces interactions et l'enseemble des réactions cellulaires dont les hormones sont à l'origine. Les chercheurs oeuvrant dans ce domaine devront adopter une approche beaucoup plus vaste tenant compte de l'ensemble des facteurs, s'il désirent atteindre cet objectif.

Division of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia.

INTRODUCTION

The hypothesis to be developed in this review is that a relationship exists between the levels of hormone receptors in the liver (and therefore the resulting physiological effects of these hormones in the cell) and the levels of the mixed function oxidases responsible for metabolism of these and other compounds. In addition, it is postulated that changes in mechanism(s) of control of this interrelated system can result in significant and wide-ranging pathological effects.

In a number of species of animals there is a well documented effect of age and sex on the activity of the steroid and drug mixed function oxidases (MFO). These "sex-dependent" enzymes have been thought to be regulated by testosterone, but the details of the mechanisms involved have never been clarified. Various studies in this area have centered on neonatal androgen-induced imprinting, on control by estrogens or a postulated pituitary hormone "feminotropin", and on growth hormone. However, a major problem with all of these studies is that only one, or at most a few, of the possible control points have been measured, while major changes are known to occur in others because of the delicate feedback controls of the hypothalamic-pituitary-end organ system. This is a vital point, because it has been shown that the effects of hormones on the sex-dependent enzymes are not seen in hypophysectomized animals. There is an impressive body of evidence to show that sex hormones have a direct effect at the hypothalamic and pituitary level to modulate the release of various pituitary hormones. These hormones may exert a regulatory role on the levels of the hepatic MFO system. On the other hand, a number of workers have found various specific high-affinity, low-capacity binding "receptors" in the liver itself which appear to be regulated by sex hormones or by pituitary hormones. These receptors are undoubtedly regulators of the synthesis of various proteins, and could be involved in the MFO system as well.

Therefore, it is becoming apparent that the homeostasis of the MFO system that oxidizes endogenous compounds such as sex steroids is an exquisitely modulated, complicated system controlled by the hypothalamic-pituitary axis. Any disruption of this system by exogenous chemicals may have dramatic effects. If the timing of the exposure to the xenobiotic is during the early neonatal imprinting period, irreversible damage may occur. For example, such pathological conditions as decreased fertility or pseudohermaphrodism may be caused by disruption of the control mechanisms by exogenous chemicals. The metabolism of other xenobiotics, particularly the activation of carcinogens, is also likely to be affected. If chronic exposure to an inducer of the MFO system occurs in the adult, major changes in general cellular metabolism may also occur.

The following is a review of the effects of androgens and estrogens on hepatic MFO enzyme activities, the modulation of these effects by cellular receptors, the role of the hypothalamic-pituitary axis in this process, and the results of pharmacological intervention.

THE ROLE OF ANDROGENS AND ESTROGENS IN THE MODULATION OF HEPATIC MICROSOMAL DRUG AND STEROID-METABOLIZING ENZYME ACTIVITIES

It is well documented that sex differences in the rate of cytochrome P-450-dependent drug and steroid metabolism exist in certain species, including man (Conney 1967; Guildicell and Tillement 1977; Kato 1974; Kato and Gillette 1965a,b; Quinn et al. 1958; Pfaffenberger and Horning 1977). In the rat, the oxidative cytochrome P-450-dependent enzyme activities are usually higher in the male than in the female. Such differences have been related to effects of the sex steroids themselves, and can be used as a model system to study control mechanisms.

INDIRECT EVIDENCE

During the immature period (first 4 weeks of age) enzyme levels in male and female rats are very low and quite similar. As male rats mature (4-8 weeks), enzyme activities gradually increase (El Defrawy El Masry et al. 1974; Henderson 1971). Coincidently, testosterone levels increase at the same rate (Forest 1979). In female rats, the enzyme activities appear to decrease slightly with maturation (El Defrawy El Masry et al. 1974). These data suggest that androgen induces and estrogen suppresses MFO enzyme activities.

DIRECT EVIDENCE

Castration of adult male rats results in a decrease in sex-dependent enzyme activities, and this alteration can be reversed by the administration of testosterone (El Defrawy El Masry and Mannering 1974; Gurtoo and Parker 1977; Kato and Gillette 1965b), suggesting that androgens are necessary to preserve a typical metabolism in the adult male. Estrogen administered to normal male rats decreases cytochrome P-450-dependent enzyme activities to female levels (Sweeney and Cole 1980). We have also shown this to occur with the synthetic estrogen, mestranol (Table 1).

Estrogen administered to castrated male rats further decreases the enzyme activities to the female level (Kramer et al. 1978; Kramer and Colby 1976). These authors suggested that a part of the sex difference was due to estrogen suppression. However, whether this is an indirect anti-androgenic effect or a direct estrogenic effect is not yet established.

Ovariectomy of adult female rats with or without estrogen replacement has little effect on hepatic oxidative drug or steroid metabolism, suggesting that estrogens do not appear to be necessary to preserve the female pattern of metabolism in the adult, i.e. that

Table 1. Effect of pretreatment of male Wistar rats with mestranol (1.5 µmole/kg subcutaneously for 14 d) on hepatic microsomal enzyme activity (n=4; ± SE).

	Benzo(a)pyrene Hydroxylase pmol/min/mg protein	Ethylmorphine N-Demethylase nmol/min/mg protein	Aminopyrine N-Demethylase
Control male	4108 ± 285	3.31 ± 0.28	4.35 ± 0.09
Mestranol-treated males	518 ± 61	0.85 ± 0.03	1.60 ± 0.11
Control females	342 ± 26	0.58 ± 0.08	0.80 ± 0.04

permanent imprinting may have occurred (Al-Turk et al. 1980; Gurtoo and Parker 1977).

PERMANENT IMPRINTING OF RESPONSIVITY TO ANDROGENS

Chung et al. (1975) reported that male rats castrated at birth respond less to androgen stimulation as adults than if exposed to androgens neonatally, as measured by aminopyrine and ethylmorphine metabolism. Denef and De Moor (1972) have shown that castration of male rats at birth will remove the sexual differentiation of cortisol metabolism which normally occurs. However, if the males were castrated on day ten of life, normal metabolic differentiation was observed. These investigators have also demonstrated that administration of a single dose of testosterone on day one of life to the castrated male or female neonate would produce a masculinization of cortisol metabolism in the adult animal (Denef and De Moor 1968). Furthermore, using the androgen antagonist cyproterone acetate, Denef and De Moor (1972) have shown that normal metabolic differentiation is inhibited in the male. These studies indicated that the earlier the male rat was isolated from androgen exposure, the more feminized the enzymatic pattern will become. In addition, it was suggested the mammalian liver is initially "feminine", and that the characteristic male enzymatic activities first seen post-pubertally are predetermined by a testicular factor neonatally. The effect of androgen exposure during the neonatal period resulting in differentiation at maturity is referred to as neonatal androgen-induced imprinting (Chung 1977; Einarsson et al. 1973). The imprinting of steroid metabolism has been extensively reviewed by Skett and Gustafsson (1979).

In addition, De Greef et al. (1980) found that neonatally castrated male rats implanted with ovarian grafts at 80 days of age were able to release prolactin in a diurnal surge-like pattern. Adult castrated males were unable to do this, presumably because of neonatal androgen imprinting.

As would be predicted from the studies of male rats, adult females (intact or ovariectomized) are relatively insensitive to androgens (Kato et al. 1968; Kato and Onoda 1970; Berg and Gustafsson 1973; Finnen and Hassal 1980). We have confirmed this using relatively low doses of testosterone and short time periods of administration (Table 2). Lack of exposure to the neonatal surge of androgen is one reason for this insensitivity. However, it is more complex than that. For example, during the time period in which puberty normally occurs, neonatally castrated rats are capable of responding fully to testosterone challenge (Chung et al. 1975). In addition, the presence of estrogen during puberty appears to have profound influences on the responsivity of enzymes to sex steroids in the adult. Estrogen treatment from day 12-32 to male rats castrated at day 12 (i.e. already imprinted with neonatal androgen) completely feminized the corticosteroid metabolism pattern, and permanently abolished the response of these

Table 2. Effect of gonadectomy and testosterone enanthate (5μmol/kg subcutaneously of 10 d) on hepatic microsomal enzyme activity in Wistar rats (n=4; ± SE).

	Benzo(a)pyrene Hydroxylase pmol/min/mg protein	Ethylmorphine N-Demethylase nmol/min/mg protein	Aminopyrine N-Demethylase nmol/min/mg protein
Males			
sham-operated controls	1240 ± 69	1.19 ± 0.10	1.58 ± 0.15
gonadectomized	642 ± 40	0.68 ± 0.08	0.89 ± 0.03
gonadectomized plus testosterone	1919 ± 148	2.46 ± 0.20	2.45 ± 0.13
Females			
sham-operated controls	342 ± 26	0.58 ± 0.08	0.80 ± 0.04
gonadectomized	223 ± 18	0.36 ± 0.05	0.58 ± 0.02
gonadectomized plus testosterone	580 ± 89	0.59 ± 0.02	0.84 ± 0.06

animals to subsequent testosterone treatment (Denef and De Moor 1972). Estrogen treatment from day 45-60 to males castrated at day 12 also feminized corticosteriod metabolism, but subsequent testosterone therapy restored the masculine pattern. Therefore, the timing of treatment is very important, and depends on the sex of the animal.

It would appear logical to conclude that many of the effects described above could be due to changes in steroid receptor number or affinity, since the actions of the hormones are mediated via cytoplasmic receptors.

ANDROGEN RECEPTORS

The present concept of steroid hormone action holds that the interaction between hormone and specific cytoplasmic receptor protein represents a critical initial step in their mechanism of action (Gorski and Gannon 1976). It has been suggested that the lack of response to testosterone in neonatally castrated male rats or in normal female rats may be due to the lack of induction of an hepatic androgen receptor (Kato 1974). Although hepatic androgen receptor mediating MFO enzyme synthesis has not been proven, there is a great deal of suggestive evidence for it. Gustaffson et al. (1975) reported an uptake of both radio-labelled testosterone and its metabolites into cytosolic and nuclear fractions of livers taken from male and female rats. Radiochromatographic analysis of cytosolic fractions of castrated male rat livers indicated three types of labelled fractions: a high affinity, low capacity ^{3}H-androstenedione binding protein, a fraction containing protein-bound ^{3}H-testosterone and monosulfates, and another fraction with unbound metabolites. Unfortunately, a similar type of analysis of female liver cytosol was not reported in this study, so it is uncertain if the absence of cytosolic androgen receptor in animals of this sex contributes to the lack of testosterone effect, as compared with possible estrogen receptor or nonspecific binding site mechanisms. Brown et al. (1976) were able to show that flutamide, a non-steroid androgen antagonist, blocked the testosterone-induced increase in ethylmorphine N-demethylase activity in castrated BALB/cJ mice and in castrated and intact prepubertal male Fisher rats. These effects seemed to be quite distinct from flutamide's nonspecific action on liver weight and microsomal protein content, which appeared to be independent of an androgen receptor.

The role of an androgen receptor on hepatic drug-metabolizing systems has also been studied using the Stanley-Gumbreck pseudohermaphroditic rat (Stanley et al. 1973). It is thought that this animal lacks a functional androgen receptor since it does not respond to pharmacological doses of testosterone (Bardin et al. 1973), even though similar doses of the same drug reduced hexobarbital sleeping times, and increased ethylmorphine N-demethylase activities in both intact females and castrated male rats (Bullock et al. 1971). Alternatively, one may postulate that the pseudohermaphroditic rat may have a defect at intracellular loci

other than an androgen receptor, which results in a faulty enzyme induction system. Sonawane et al. (1979) has recently reported that phenobarbital can stimulate aminopyrine N-demethylase and aniline hydroxylase activities in male, female and pseudohermaphroditic rats. We have confirmed and extended these results (Fig. 1). Therefore, it appears that the enzyme induction mechanism remains intact, but that the phenobarbital (PB)- and testosterone-type enzyme induction is mediated at least at some stage by two separate regulatory points. Though the effects of testosterone may be similar to the classical enzyme inducer class typified by PB, it differs in a number of ways. This suggests the possibility of an androgen receptor. A cytosolic receptor for PB has not been found, but may exist. From the results of Poland et al. (1976), induction of certain enzymes involves a receptor ligand complex. Therefore, enzyme induction might be defined and characterized at the receptor level by the spectrum or profile of enzymes being activated. These observations would suggest that the multiplicity of different forms of cytochrome P-450 can be postulated to be associated with a diversity of receptors, either present in the cytoplasm or elsewhere in the cell. In general, the results mentioned above suggest an intimate relationship between androgen, its receptor and enzyme induction as first postulated by Kuntzman et al. (1964).

A systematic study of how steroid receptor involvement regulates drug metabolism has not been pursued. Though there is evidence to suggest a regulatory role of cytosolic androgen receptors in extrahepatic target tissue, drug metabolism research has previously been limited by the failure to demonstrate the existence and correlated physiological action of a specific hepatic androgen receptor in vivo and in vitro. Ligandin, one of many forms of the soluble enzyme glutathione S-transferase, has been found to bind many compounds intracellularly, including sex steroids such as testosterone and estrogen in hepatic tissue (Ketterer et al. 1975, 1976), but Smith and Litwack (1980) doubt that ligandin has any direct involvement in steroid effects. They noted that the tissue distribution of ligandin does not correlate with the tissue potency of the hormonally active steroids (Katzellenbogen and Gorski 1975; Mainwaring et al. 1973), nor does it have a high affinity for the ligand (range 10^{-4} to 10^{-5} M). The low affinity, high capacity characteristics of ligandin may lead one to associate it to an intracellular reservoir for steroids and their metabolites rather than to a sensitive control point for hormonal signals. If so, it may regulate the intracellular concentration of active free hormone, this would be analogous to drug binding plasma proteins which affect serum free drug concentrations. If ligandin serves this type of purpose, then drug interactions resulting from alterations of drug binding capacities may be a phenomenon common to both intra- and extracellular drug binding proteins.

Further evidence that a specific (high affinity, low capacity) androgen receptor may exist in rat liver was provided by Fang and Liao (1971). They mixed radioactive dihydrotestosterone (DHT) in vitro with either whole cytosol or cytoplasmic DHT binding protein from rat ventral

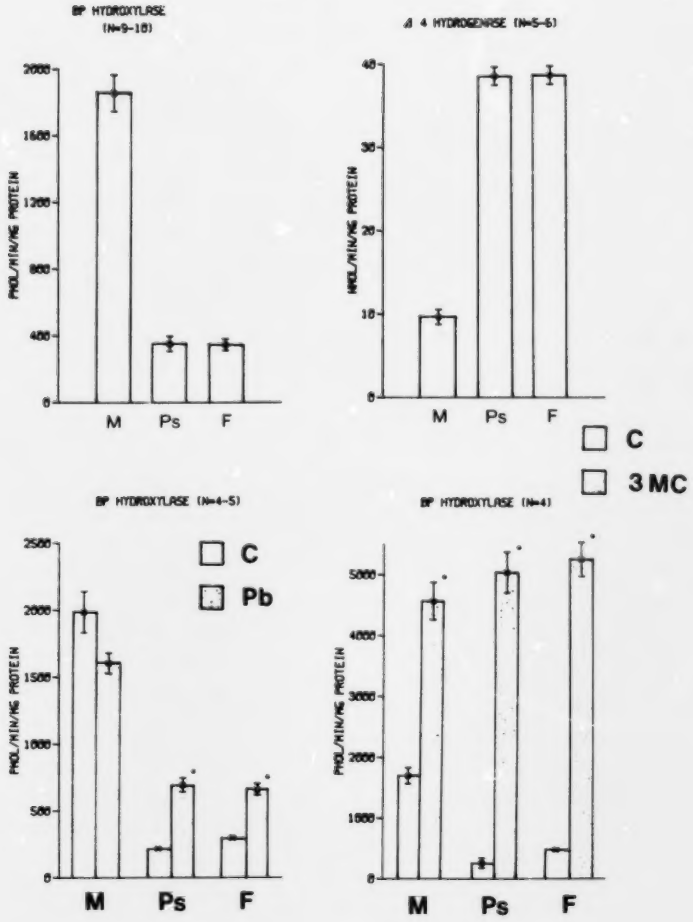


Figure 1. Hepatic microsomal enzyme activities in the pseudohermaphroditic (Ps) rat as compared to its male (M) and female (F) controls. Pb = treated with 80 mg/kg/d phenobarbital sodium i.p. for 2 d; C = control (vehicle treated). mean ± SE; * = significantly different from control levels at $p < 0.05$.

prostate. These fractions were then incubated with purified hepatic nuclei. When the labelled steroid protein complex was isolated from the nuclei, the liver nuclei retained more radioactive DHT than did nontarget tissue nuclei in blood, spleen, lung, thymus, and diaphragm. The work of Gustaffsson et al. (1975) also supports the hypothesis that an androgen receptor is present in liver tissue. Radiolabelled testosterone was injected i.p. to castrated male rats. (Castration causes androgen receptors to be localized in the cytoplasm, since binding of endogenous androgen ligand and subsequent transport to the nucleus no longer occurs.) The liver cytosol obtained from these rats contained a protein-labelled androstenedione complex. This complex had a sedimentation coefficient of 10 S, was destroyed by incubation with pronase, and was stable at 37°C for at least 60 min. Similar androstenedione binding was observed in vitro where unlabelled cytosol from castrated rats was incubated with labelled testosterone. The data indicated that under these experimental conditions testosterone had been metabolized to androstenedione. The authors interpreted the findings to suggest the existence of specific mechanisms in liver cytosol which produce androstenedione from testosterone, protect it from further metabolism, and provide for its uptake into liver nuclei. However, there were no data presented to indicate the specificity of the androstenedione binding protein. This would have been of interest since Roy et al. (1974) previously reported the existence of a DHT binding receptor in liver cytosol. The receptor was characterized to be a protein with a sedimentation coefficient of 3.5 S. It bound DHT, testosterone, and 17β-estradiol with high affinity. Specific binding of DHT to the receptor had a K_d of 4.5×10^{-8} M; and the K_d for estradiol binding to the receptor was 3.5×10^{-7} M. No data were presented regarding nuclear binding of the DHT-receptor complex. These workers correlated the existence of the androgen receptor in hepatic cytosol with the urinary output of α_{2u}-globulin, a protein synthesized in the liver under the influence of androgen, and which appears in the urine of male rats. Immature male rats and female rats did not produce α_{2u}-globulin and were found to lack this liver androgen receptor activity as well. Castration or estradiol treatment of male rats led to the loss not only of cytoplasmic androgen receptor activity but also of α_{2u}-globulin synthesis. These data indicated that the cytoplasmic androgen receptor was induced by androgens and was suppressed by estradiol. Another significant finding was that pseudohermaphroditic male rats which show androgen insensitivity at the end organ level, did not synthesize α_{2u}-globulin (Milin and Roy 1973). In turn, these animals were found not to show any androgen receptor activity, indicating further the correlation between the liver cytosol androgen receptor and hepatic synthesis of α_{2u}-globulin.

Recently, another group has found a testosterone- and androstenedione-binding protein in male rat liver (Sato et al. 1980a, 1980b; Ota et al. 1980). This cytosolic component exhibited a sedimentation coefficient of 10 S, a dissociation constant for testosterone of 1.37×10^{-7} M and a capacity of 313 fmols/mg protein. Both cytosolic and nuclear binding sites were decreased following

castration, and increased by testosterone. Estradiol-17β did not appear to compete with testosterone in the *in vitro* binding experiments.

In summary, there is suggestive evidence that an androgen receptor exists in rat liver which is related to sex-hormone status, and that its presence is correlated with certain physiological endpoints (e.g. synthesis of $\alpha_{2\mu}$-globulin) and pharmacological endpoints (e.g. induction of MFO by testosterone).

ESTROGEN RECEPTORS

The existence of a hepatic estrogen receptor in rats was recently demonstrated by 4 groups of workers (Beers and Rosner 1977; Chamness *et al.* 1975; Eisenfeld *et al.* 1976; Viladiu *et al.* 1975). Beers and Rosner (1977) detected a saturable estrogen binding 4 S protein in liver cytosol with an association constant of 5.98×10^9 L/mol. Eisenfeld *et al.* (1977) found that estrogen binding in rat liver was lower than that found in uterus, but was higher than in plasma, spleen, and heart. The receptor showed specificity: estrone, estriol and mestranol bound to it, whereas DHT, testosterone, progesterone, cortisol and dexamethasone did not. It was shown that the hormone-receptor complex was also able to translocate to the nucleus. Therefore, the liver appeared to contain a protein which bound estradiol with high affinity and specificity. These properties and sedimentation characteristics were essentially similar to those of the estrogen receptor identified in rat uterus. In addition, Eisenfeld's group has been able to correlate estrogen receptor levels in rat liver with the hepatic synthesis of a plasma protein. "Plasma renin substrate" levels and estrogen receptor levels were elevated in adult and estrogen-treated female rats but not in prepubescent female rats (Eisenfeld *et al.* 1976, 1977).

Estrogen receptors are present not only in female rat liver but also in male rat liver (Aten *et al.* 1978). In male rat liver there appear to be at least two types of estrogen binding proteins: a moderate affinity and moderate capacity protein, and a high affinity low capacity receptor protein. The high affinity, low capacity receptor has similar properties in male and female rats. However, the less specific receptor additionally present in male rat liver is present in much smaller amounts in immature male and female rats and is low to nearly absent in mature females (Powell-Jones *et al.* 1980). This group also noted that castration decreased the levels of this receptor in the male by only 20%, although Dickson *et al.* (1978) found approximately a 50% decrease. From the work of the previously mentioned groups, along with several other reports (Dickson *et al.* 1980; Dickson and Eisenfeld 1980; Mulder *et al.* 1974), the properties of the two types of estrogen binding proteins may be defined (Table 3). It should be noted that there are certain striking similarities between the moderate affinity estrogen binding protein and the androgen receptor defined by Roy's group.

Table 3. Properties of estrogen binding proteins.

Source	Female Rat Liver	Male Rat Liver
A) High Affinity Estrogen Binding Protein		
K_D	0.7-1.7 x 10^{-10} M	1.4 x 10^{-10} M
K_D (ammonium sulfate ppt)	2.2 x 10^{-10} M	3.5 x 10^{-10} M
capacity	14-58 fmol/mg protein	2.3 fmol/mg protein
capacity (ammonium sulfate ppt)	25 fmol/mg protein	25 fmol/mg protein
typical compounds which compete for binding sites	estradiol, diethylstilbestrol, estrone, estriol, ethinylestradiol	estradiol, diethylstilbestrol, estrone, ethinylestradiol
typical compounds which do not compete for binding	dihydrotestosterone, testosterone, progesterone, cortisol, corticosterone	dihydrotestosterone, progesterone, corticosterone
sedimentation coefficient	8-9 S	8 S
source	female rat liver	male rat liver
evidence for nuclear translocation and binding	yes (mainly unchanged estradiol bound)	yes - but lower onset of translocation, longer nuclear retention (mainly a polar metabolite bound)
effect of age	low levels in immature animal at puberty, high levels develop	low levels in immature animal
effect of castration	1 day - slight decrease 2 weeks - 2 ½-fold increase	3 weeks - 30% decrease
effect of hypophysectomy	decreased binding	decreased binding
B) Moderate Affinity Estrogen Binding Protein		
K_D		0.6-1.3 x 10^{-7} M
capacity	(10-200-fold less than present in adult male)	0.48-1.8 pmol/mg liver
typical compounds which compete for binding sites		estradiol, estriol, ethinyl, estradiol, dihydrotestosterone, 5α-androstan-3α,17β-diol
typical compounds which do not compete for binding sites	diethylstilbestrol	diethylstilbestrol, corticosterone, cortisol, progesterone
sedimentation coefficient	4-5 S	4 S
evidence for nuclear translocation	no (remains in cytoplasm)	no (minimum translocation of estradiol at short time periods; increased after longer time periods)
source	female rat liver	male rat liver
effect of age	little	adult has 200-fold greater amount than immature male (main binding component of adult liver)
effect of castration		20-50% decrease
effect of hypophysectomy		decreased to female level; no response to estradiol treatment

Recently, various inducers of the hepatic mixed function oxidases have been shown to decrease cytosolic estrogen binding in ovariectomized rats (Duvivier et al. 1981). For example, one 40 mg/kg dose of 3-methylcholanthrene (3-MC) decreased binding by 44% after 24 h and 80% after 72 h. The authors found an increase of 17% 24 h after a 30 mg/kg dose of phenobarbital. However, the discrepancy between the low dose and short time period in relation to maximal enzyme induction by PB, in contrast to the supramaximal dose of 3-MC calls into question any conclusion about the phenobarb results. Further studies with higher doses over a longer time period would clarify this if a difference in pattern does exist. However, the findings with the other inducers are of considerable importance. In particular, these authors found an excellent negative correlation between the effects of 3-MC or benzo(a)pyrene on cytosolic estrogen receptor and aryl hydrocarbon hydroxylase activity. Since the estrogen binding changes were not observed in other tissues, such as uterus, and were not accompanied by a decrease in lactate dehydrogenase (another cytosolic protein), the effect does not appear to be a general nonspecific one.

These findings support the hypothesis that some of the effects of monooxygenase inducers are mediated by a specific cytosolic receptor. It was postulated that because of structural similarities between the polycyclic hydrocarbons and estrogens, these inducers or their electrophilic metabolites could interact specifically with the estrogen receptor and produce a modification of the estrogen binding sites. Alternatively, it was suggested that one of the consequences of the nuclear effects of the polycylic hydrocarbons, besides that of induction of the monooxygenases, could be a decrease in synthesis of cytosolic estrogen receptors. In any case, the end result is that treatment with exogenous compounds causes profound changes in the normal physiological state with respect to hepatic cytosolic estrogen receptors.

In summary, it is evident that rat liver contains various steroid receptors. These steroid receptors may interact to modulate the overall metabolic response of the animal, in particular, to direct the type and amount of synthesis of specific proteins. Furthermore, the receptor levels appear to respond to various treatments in a way that is correlated with the response of sex-dependent MFO enzyme activities. Therefore, the hypothesis that steroid receptor levels are involved in control of or modulation by sex dependent metabolizing enzymes is supported.

INDIRECT EFFECTS OF SEX HORMONES ON HEPATIC MIXED FUNCTION OXIDASES

Although it would appear possible that one site of action of the sex steroids on the hepatic MFO is directly on the liver, there is evidence that at least part of their action is indirect. The previously described effects of testosterone or estradiol on the "sex-dependent" hepatic MFO are not seen in hypophysectomized animals and, in addition,

androgenic neonatal imprinting of these enzymes has been reported to be dependent on an intact pituitary gland (Burke et al. 1978; Chung 1977; Denef 1974; Gustafsson and Stenberg 1974; Kramer et al. 1975a; Kramer et al. 1979; Lax et al. 1974). When pituitary glands from male or female rats over 35 days old are transplanted under the renal capsule (so that they still secrete hormones but are not under direct hypothalamic control), a "feminized" pattern of enzymes is produced (Gustafsson and Stenberg 1976; Gustafsson et al. 1977; Gustafsson et al. 1978; Gustafsson and Skett 1978; Skett et al. 1978). This led Gustafsson to postulate the existence of a new pituitary hormone, "feminotropin", since they could not achieve similar effects using any of the known pituitary hormones, i.e. prolactin (PRL), growth hormone (GH), thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), or luteinizing hormone (LH). Colby's group has also shown that treatment of hypophysectomized male rats with FSH and/or LH, or PRL produced no "feminizing" effects on the hepatic Δ^4-hydrogenases or MFO (Colby et al. 1974). The direct effect of FSH or LH in gonadectomized male rats was a slight induction, e.g. ethylmorphine metabolism (Kramer et al. 1977). This group also found that the effects of thyroxine and corticosteroids on hepatic mixed function oxidases were qualitatively similar with or without the presence of the pituitary (Rumbaugh et al. 1978). Kramer et al. (1975b) and Rumbaugh and Colby (1980) showed that in hypophysectomized animals, a combination of GH, thyroxine and ACTH simulated the "feminizing" effects of GH or estradiol in normal male rats. They concluded that GH is the pituitary factor necessary for the "feminizing" effects of estradiol on the MFO enzymes and that thyroxine and ACTH influence this activity. However, there are a number of weaknesses in the experiments leading to this conclusion. Early work measuring PRL or GH levels is suspect because of the nonspecificity of the assays. Also, early PRL and GH preparations were not pure. Most importantly, hypophysectomy causes extensive changes in many parameters. Most workers had supplemented the animals with only one hormone, leaving gross metabolic abnormalities in many others. In the work by Colby's group (1974), a great deal of attention was paid to attaining results with GH in combination with other hormones, but that cannot be said for the data on PRL, or other hormones. This is important because PRL is a major controlling enzyme in the release of LH releasing hormone (LHRH) from the hypothalamus, because PRL receptors in the liver are under estrogenic control, and because some of the data used to support the role of GH were derived using human(h)-GH which possesses lactogenic activity.

A high specificity PRL receptor is also present in female rat liver which increases during pregnancy (Posner et al. 1974, 1975). Very low levels of receptors are present in liver of prepubertal males or females, or in the adult male, but increased receptors can be induced by estradiol or estrone. This induction takes 8-12 days to achieve maximal effect. After hypophysectomy, receptor levels are decreased, and the inductive effect of estradiol is blocked in both males and females. Implantation of the pituitary under the renal capsule induces PRL receptors several days after increased serum PRL. Further studies have led this group to postulate that a combination of PRL, GH and ACTH

induces lactogenic receptors in rat liver, and that this effect is modulated by sex hormones (Bohnet et al. 1976; Aragona et al. 1976).

Recently, this work has been extended by a Japanese group (Furuhashi and Fang 1979), using a GH and PRL-secreting tumor (GH_3). They found an increased number of specific membrane binding sites for h-PRL concomitantly with increased GH and PRL. The affinity constant was not changed. Male rats exhibited greater increases than did females. Positive correlations between both serum GH and PRL levels, and PRL binding sites, were found in females, with a positive correlation between binding and GH in males. A significant negative correlation between PRL binding and serum testosterone was found in male rats. Corticosterone and estrogen showed no correlation with PRL binding. They conclude that although serum GH and PRL regulate PRL receptors in adult female rat liver, an inhibition of induction of PRL receptors by testosterone is the major regulatory factor in adult male rats (overcoming any stimulatory effect of physiological estrogens). Exogenous GH was confirmed to be the most effective inducer of lactogenic binding sites. The authors also speculate that although it is possible that the regulatory function of sex steroids on the action of pituitary hormones is mediated at the level of the central nervous system (i.e. the hypothalamus), in these experiments the GH_3 tumor was not subject to central control. They conclude that this provides evidence for at least part of steroid regulation directly at the level of the liver.

Central regulation of PRL by sex hormones is well known (see Prolactin Symposium 1980). Estradiol increases the number of binding sites for PRL releasing factor in the anterior pituitary, and decreases dopamine binding sites. Both these effects result in increased PRL release in both males and females. Excess PRL decreases the release of LHRH from the hypothalamus by negative feedback. Since androgens also decrease LHRH at the hypothalamic level, the two hormones appear to act in conjunction. Estrogen also effects the activity of GH by decreasing somatomedin production by the liver. However, it has recently been shown that the number of GH receptors in rat liver is very low, and that both males and females have similar amounts (Hughes and Hughes 1981). The effects of androgens on growth are thought to be independent of somatomedins. In summary, there is evidence pointing both to central and local effects of sex hormones on the control of either levels or activity of PRL and GH.

Furthermore, the study of the effects of exogenous opiates or endogenous opioid peptides on the secretion of hormones from the pituitary has provided evidence for the effect of sex hormones on the central control of PRL and GH release (see Opiate Symposium 1980). Endogenous opioid peptides or exogenous opiates act to inhibit the release of dopamine by the dopaminergic cells of the hypothalamus. This decreases the inhibitory influence of dopamine on the release of PRL and GH by the anterior pituitary. Naloxone produces opposite effects and competitively inhibits the effects of the peptides or opiates. It should

be noted that the response of GH to opiates remains during chronic therapy (Kokka et al. 1973).

It is also well known that opiates decrease serum testosterone and this appears to be due solely to a direct effect on the hypothalamus (Cicero 1980). Although endogenous opiates participate in testosterone's negative feedback control of LH at the hypothalamus, testosterone and naloxone do not compete with each other for common binding sites in this central region (Cicero et al. 1979, 1980). LaBella and Pinsky found many common properties of androgens and narcotics, and observed displacement of testosterone by opiates in liver microsomes, though at very high concentrations (Pinsky et al. 1975; LaBella 1975; LaBella et al. 1977). Hahn and Fisherman (1979) noted a twofold increase in saturable binding of naltrexone in brains of castrated male rats compared with those of controls. This was decreased to normal levels by testosterone administration. The increased content of opiate receptors correlated with an increase in brain N-demethylation of morphine. Microsomes from rat brain do possess a cytochrome P-450 MFO system which N-demethylates various compounds and which is inhibited by Δ^4-androstene-3,17-dione. This suggests a possible interaction between endogenous steroid hormones and opiates at the metabolic level in the central nervous system (Marietta et al. 1979). We have found that opiates produce significant effects on the hepatic MFO enzymes (Bellward et al. 1977a, 1977b; Gontovnick and Bellward 1980).

In summary, there is evidence that a combination of pituitary hormones, including PRL, GH, ACTH and thyroxine, may be controlling the activity of various drug and steroid MFO enzymes in the liver. The effects of these hormones are modified by the sex steroids, apparently both at central and peripheral levels, and conversely, the effects of the steroids are modified by the levels of pituitary hormones and their hepatic receptors.

LONG-TERM CHANGES IN DRUG AND STEROID MFO ACTIVITY

We have found that low doses of 1-α-acetylmethadol administered via the drinking water to female Wistar rats throughout pregnancy and lactation produced long-term changes in the activites of various hepatic MFO systems in the adult offspring (Finlayson and Bellward 1981). For example, there was a increase in apparent K_m for benzo(a)pyrene hydroxylase over control levels in both males and females. The apparent K_m for aminopyrine N-demethylase in males was decreased by perinatal treatment to control female levels, whereas it was increased above control male values in the treated female offspring.

Permanent alterations in reproductive function have been shown to occur in the offspring of rats exposed to 40 mg/kg/d of PB during days 12-19 of pregnancy (Gupta et al. 1980). Fertility of males was decreased to 64% of control, and this was accompanied by a significant decrease in plasma concentrations of testosterone and LH. Liver weight was slightly

increased and seminal vesicle weight decreased in the adult offspring of the PB-treated dams. Cytoplasmic androgen binding in the seminal vesicles was increased over control values. Androgen binding was not measured in liver, but no changes were found in prostate and brain tissue.

In a study by Soyka (1980) on pregnant Swiss-Webster mice, a single dose of 4 mg per mouse of benzo(a)pyrene was administered subcutaneously at various stages of gestation (days 3-18). The offspring were studied when they were 2-3 months old to examine for evidence of certain long-lasting effects, including increased susceptibility to cancer. In the treated females, hepatic aminopyrine demethylase was higher and cytochrome P-450 was slightly decreased in the male treatment group. Using intramuscular injections of 3-MC to produce either local or lung tumor in the adults, the authors found a further sex difference. The benzopyrene offspring developed local tumors at an earlier time period than the controls, and this was most apparent in the females. In addition, these females exhibited a higher incidence of both local tumors and lung adenomas. No liver tumors were found in this study.

It should be noted that 1-α-acetylmethadol, PB and benzo(a)pyrene are all inducers of the hepatic MFO system. It is conceivable that exposure to such chemicals during critical perinatal time periods will permanently alter the number or affinity of various important receptors either locally in the liver, or elsewhere, e.g. in the hypothalamus or pituitary.

MULTIPLE FORMS OF P-450

It is now well known that multiple forms of P-450 exist (Lu and West 1979). In order to overcome the problem of interpretation inherent in using microsomal preparations containing many forms of cytochrome P-450, Harada and Omura (1981) employed quantitative immunochemical techniques. They prepared purified P-450 from liver of PB-treated or 3-MC-treated rats, raised the corresponding antibodies in rabbits, purified the immunoglobulins and utilized them to quantitatively precipitate the specific proteins. It was found that each of the forms of P-450 induced by PB or by 3-MC in control Sprague-Dawley rat liver accounted for about 10% of the total P-450. When the animals were pretreated with one of these inducers, however, the pattern rapidly changed. After 24 h, the total P-450 had approximately doubled in either case, whereas the form corresponding to the inducer had increased by 7 to 10-fold. Further, the amount of the other form of P-450 measured did not change, i.e. the induced form selectively became the quantitatively predominant P-450.

The results of studies on the substrate specificity of these forms of P-450 are of particular interest. In microsomes from untreated animals, it appeared that the PB-inducible form was responsible for the majority of the drug oxidation reactions, even though it accounted for only 10% of the P-450. It catalyzed 98% of the total benzphetamine

N-demethylation, 54% of the 7-ethoxycoumarin O-deethylation and 74% of the benzo(a)pyrene 3-hydroxylation, whereas the 3-MC form metabolized 20% of the 7-ethoxycoumarin and none of the other two substrates.

Large changes in substrate specificity were noted following drug treatment. For example, 48 hours after a 3-MC injection, the P-450 induced by this compound virtually dominated the metabolism of 7-ethoxycoumarin and benzo(a)pyrene, while the metabolism of benzphetamine continued to be carried out by the PB form. It should be emphasized that these results do not agree with data from experiments using reconstituted enzymes, a finding also noted by Elshourbagy and Guzelian (1980) with the form of P-450 induced by pregnenolone-16α-carbonitrile. It appears that the substrate specificity may be altered during the purification procedures, or that other components of the redox system present in the microsome are necessary for normal activity.

Harada and Omura (1981) carried their experiments one step further. After maximally inducing animals with PB, they added a single injection of 3-MC. Twenty-four hours later, when the level of PB-induced P-450 had not changed, virtually all the 7-ethoxycoumarin and benzo(a)pyrene was metabolized by the 3-MC-induced form of the enzyme; i.e. an additive effect was not seen. This finding has very significant implications with regard to interpretation of data from previous studies, where the contributions of the individual forms of P-450 to drug metabolism are not known. In contrast to the above two substrates, the PB-form of P-450 continued to fully metabolize benzphetamine, even in the 3-MC-induced liver. The authors suggest that such inducer therapy may "result in significant alterations in the electron flow from NAD(P)H to various forms of cytochrome P-450, affecting their oxidation activities". However, it could also be postulated that conformational changes in specific binding sites would produce such results. It will be most important to determine what happens to endogenous substrate enzyme specificities following induction by various drugs.

PROMOTION OF HEPATOCELLULAR CARCINOMA

It is known that inclusion of 0.05% PB in the diet of rats following the administration of an initiator such as 2-acetylamino-fluorene produces a 10-fold increase in hepatocellular carcinomas. Pitot (1978) has confirmed this promoter effect of PB using the system of diethylnitrosamine and partial hepatectomy. Foci of hepatocytes with altered enzyme activities have been postulated by Pitot and Sirica (1980) to be precursors of hepatocellular carcinomas. Certain enzymes, e.g. glucose-6-phosphatase and canalicular ATPase, are found to be decreased in these foci, whereas γ-glutamyl transpeptidase and epoxide hydrase are elevated. In addition, these cells exhibit an increased mitosis and synthesis of DNA. PB has also been noted to double the incidence of "spontaneous" hepatomas in male C3H mice (Peraino et al. 1973a,b) which

implies that promotion of underlying "spontaneous" neoplasms would be expected to occur in other situations too.

Pitot and Sirica (1980) have indicated the similarities in the pathogenesis of liver cancer in experimental animals and humans. In particular, the promoting effect of ethanol on hepatocellular carcinoma is of interest. Although there is apparently no evidence at present that PB by itself causes an increased incidence of liver cancer in humans, there may be an effect on certain brain tumors (Gold et al. 1978).

As is commonly known, PB and ethanol are inducers of cytochrome P-450. Interestingly, a number of other P-450 inducers have also been shown to promote hepatic cancer in animals, notably estradiol and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The apparent mechanism of action of TCDD is via a cytoplasmic receptor (Poland et al. 1976). Poland and Glover (1979) have shown that TCDD has a negligible interaction with DNA, tending to rule out any possible initiator role for this compound.

The question that arises, therefore, is that of a cause or effect relationship between induction of P-450 and promotion of cancer. If promotion is a change in the epigenetic regulation of gene expression, one can postulate that this is mediated through an effect on cytosolic or membrane receptors. An effect on cellular hormone receptors would be expected to result in changes in the normal physiological actions of these hormones, e.g. selective increases in protein synthesis, hyperplasia, etc. Such effects would be predicted to cause widespread aberrations, thereby changing cellular responses both to endogenous and exogenous compounds.

CONCLUSION

Various drugs are known to induce specific forms of cytochrome P-450. Alternatively, there is evidence that at least some drugs may produce immediate conformational changes in the cytochrome. In either case, the presence of the exogenous compound could redirect endogenous metabolic pathways away from the norm. The result of such redirection could be a change in the metabolism of some endogenous compounds which are responsible for the control of specific protein synthesis, cell turnover, etc. Thus the buildup of the substrates or the decrease in level of metabolites, would radically change the biochemical status of the cell. The fact that the drug causing this is fortuitously metabolized and excreted may be obscuring the underlying pathological results.

It is the contention of the authors that until we understand the basic control mechanisms and interrelationship inherent in this system, we cannot logically predict drug toxicities, nor can we set up effective short-term screening assays for these toxicities. Certain

limitations of our knowledge are immediately apparent. For example, since it now appears that the control mechanisms for synthesis of the MFO enzymes differs with enzymes of different substrate specificity, it is necessary to measure the metabolism of a number of drugs in order to work out the patterns of change. Measuring blood levels of hormones or drugs is only of partial value; the number and affinity of their tissue receptors must also be known. This type of data must always be correlated with a physiological response in order to provide assurance of some reasonable biological meaning. Differences in response to drugs in relation to age, sex, pathological state etc. must be monitored. In addition, we cannot look at the liver in isolation from the rest of the body. The hypothalamus and pituitary may provide major control mechanisms for liver metabolism. Changes in amount or specificity of metabolism in nonhepatic tissue may be of much greater toxicological importance because of the lower basal levels of P-450 present in the control state. We do not understand the detailed properties of the P-450 system, nor do we have adequate methods at present to study these enzymes. Severe limitations exist in the use of purified, reconstituted systems or cultured hepatocytes in that the substrate specificities appear to be changed.

Cytochrome P-450 is a fascinating biochemical enigma. If we wish to understand the toxicological implications of this enzyme system, we must return to first principles while at the same time maintaining an overview of the whole organism. There are no compelling a priori reasons to assume that these enzymes are unique in their fundamental biological properties or that they are immune from the normal cellular control systems. Perhaps cytochrome P-450 is not, after all, the center of the universe, but simply one part of a complex, exquisitely interrelated biological system.

REFERENCES

Al-Turk, W., Stohs, S., Roche, E.B. 1980. Altered metabolism of 7-ethoxycoumarin by hepatic, pulmonary, and intestinal microsomes from streptozotocin-diabetic rats. Drug Metab. Dispos. 8: 44-45.

Aragona, C., Bohnet, H.G., Fang, V.S. and Friesen, H.G. 1976. Induction of lactogenic receptors II. Endocr. Res. Commun. 3: 199-208.

Aten, R.F., Dickson, R.B. and Eisenfield, A.J. 1978. Estrogen receptor in adult male rat liver. Endocrinology 103: 1629-1635.

Bardin, C.W., Bullock, L.P., Sharins, R.J., Mowszowicz, I. and Blackburn, W.R. 1973. Androgen metabolism and mechanism of action in male pseudohermaphrodism: A study of testicular feminization. Recent Prog. Horm. Res. 29: 65-105.

Beers, P.C. and Rosner, W. 1977. The binding of estrogens in the liver of the rat: demonstration and endocrine influences. J. Steroid Biochem. 8: 251-258.

Bellward, G.D., Gontovnick, L and Otten, M. 1977a. Induction of hepatic aryl hydrocarbon hydroxylase and expoxide hydrase in Wistar rats pretreated with oral methadone hydrochloride. Drug Metab. Dispos. 5: 211-218.

Bellward, G.D., Li, M., Lopaschuk, G.D. and Warren, P. 1977b. The effect of α-l-acetylmethadol hydrochloride on rat hepatic aryl hydrocarbon hydroxylase and epoxide hydrase. Res. Commun. Chem. Pathol. Pharmacol. 18: 415-422.

Berg, A. and Gustafsson, J.A. 1973. Regulation of hydroxylation of 5-α-androstane-3α, 17β-diol in liver microsomes from male and female rats. J. Biol. Chem. 248: 6559-6567.

Bohnet, H.G., Argona, C. and Friesen, H.G. 1976. Induction of lactogenic receptors I. Endocr. Res. Commun. 3: 187-198.

Brown, T.R., Green, F.E. and Bardin, C.W. 1976. Androgen receptor dependent and independent activities of testosterone on hepatic microsomal drug metabolism. Endocrinology 99: 1353-1362.

Bullock, L.P., Bardin, C.W., Gram, T.E., Schroeder, D.H. and Gillette, J.R. 1971. Hepatic ethylmorphine demethylase and Δ^4-steroid reductase in the androgen-insensitive pseudohermaphroditic rat. Endocrinology 88: 1521-1523.

Burke, M.D., Orrenius, S. and Gustafsson, J.A. 1978. Pituitary involvement in the sexual differentiation and 3-methylcholanthrene induction of rat liver microsomal monooxygenases. Biochem. Pharmacol. 27: 1125-1128.

Chamness, G.., Costlow, M.E., McGuire, W.C. 1975. Estrogen receptor in rat liver and its dependence on prolactin. Steroids 26: 363.

Chung, L.W.K. 1977. Characteristics of neonatal androgen-induced imprinting of rat hepatic microsomal monooxygenases. Biochem. Pharmacol. 26: 1979-1984.

Chung, L.W.K., Raymond, G. and Fox, S. 1975. Role of neonatal androgen in the development of hepatic microsomal drug metabolizing enzymes. J. Pharmacol. Exp. Ther. 193(2): 621-630.

Cicero, T.J. 1980. Effects of exogenous and endogenous opiates on the hypothalamic-pituitary-gonadal axis in the male. Fed. Proc. 39: 2551-2554.

Cicero, T.J., Schainker, B.A. and Meyer, E.R. 1979. Endogenous opioids participate in the regulation of the hypothalamic-pituitary-luteinizing hormone axis and testosterone's negative feedback control of luteinizing hormone. Endocrinology 104: 1286-1291.

Cicero, T.J., Wilcox, C.E., Bell, R.D. and Meyer, E.R. 1980. Naloxone-induced increases in serum luteinizing hormone in the male: Mechanism of action. J. Pharmacol. Exp. Ther. 212: 573-578.

Colby, H.D., Gaskin, J.H. and Kitay, J.I. 1974. Effects of anterior pituitary hormones on hepatic corticosterone metabolism in rats. Steroids 24: 679-686.

Conney, A.H. 1967. Pharmacological implications of microsomal enzyme induction. Pharmacol. Rev. 19(3): 317-366.

De Greef, W.J., Van Der Schoot, P. and Zeilmaker, G.H. 1980. Diurnal prolactin surges and sexual differentiation of the rat hypothalamus. Endocrinology 106: 486-489.

Denef, C. 1974. Effect of hypophysectomy and pituitary implants at puberty on the sexual differentiation of testosterone metabolism in rat liver. Endocrinology 94: 1577-1582.

Denef, C. and De Moor, P. 1968. The puberty of the rat liver II. Permanent changes in steroid metabolizing enzymes after treatment with a single injection of testosterone propionate at birth. Endocrinology 83: 791-798.

Denef, C. and De Moor P. 1972. Sexual differentiation of steroid metabolizing enzymes in the rat liver. Further studies on predetermination by testosterone at birth. Endocrinology 91: 374-384.

Dickson, R.B. and Eisenfeld, A.J. 1980. *In vitro* nuclear translocation of the estrogen receptor in the hepatic parenchymal cell from male rats. Mol. Pharmacol. 17: 31-37.

Dickson, R.B., Aten, R.F. and Eisenfeld, A.J. 1978. An unusual sex steroid-binding protein in mature male rat liver cytosol. Endocrinology 103: 1636-1646.

Dickson, R.B., Aten, R.F. and Eisenfeld, A.J. 1980. Receptor-bound estogens and their metabolites in the nucleus of the isolated rat liver parenchymal cell. Mol. Pharmacol. 18: 215-223.

Duvivier, J., Van Cantfort, J. and Gielen, J.E. 1981. The influence of five monooxygenase inducers on liver cytosol estradiol receptor levels in the ovariectomized adult rat. Biochem. Biophys. Res. Commun. 99: 252-258.

Einarsson, K., Gustafsson, J.A. and Stenberg, A. 1973. Neonatal imprinting of liver microsomal hydroxylation and reduction of steroids. J. Biol. Chem. 248: 4987-4997.

Eisenfeld, A.J., Aten, R., Weinberger, M., Haselbacher, G., Halpern, K. 1976. Estrogen receptor in mammalian liver. Science 191: 862-865.

Eisenfeld, A.J., Aten, R., Haselbacher, G. and Halpern, K. 1977. Specific macromolecular binding of estradiol in mammalian liver. Biochem. Pharmacol. 26: 919-927.

El Defrawy El Masry, S. and Mannering, G.J. 1974. Sex-dependent differences in drug metabolism in the rat. II. Qualitiative changes produced by castration and the administration of steroid hormones and phenobarbital. Drug Metab. Dispos. 2: 279-284.

El Defrawy El Masry, S., Cohen, G.M. and Mannering, G.J. 1974. Sex dependent differences in drug metabolism in the rat. I. Temporal changes in the microsomal drug-metabolizing system of the liver during sexual maturation. Drug Metab. Dispos. 2: 267-278.

Elshourbagy, N.A. and Guzelian, P.S. 1980. Separation, purification and characterization of a novel form of hepatic cytochrome P-450 from rats treated with pregnenolone-16α-carbonitrile. J. Biol. Chem. 255: 1279-1285.

Fang, S. and Liao, S. 1971. Androgen receptors - Steroid and tissue-specific retention of 17β-hydroxy-5α-androstan-3-one-protein complex by the cell nuclei of ventral prostate. J. Biol. Chem. 246: 16-24.

Finlayson, M.J. and Bellward, G.D. 1982. Effects of perinatal exposure of Wistar rats to α-1-acetylmethadol-HCl on hepatic microsomal mixed function oxidase enzyme activity in the adult. Drug Metab. Dispos. (in press).

Finnen, M.J. and Hassall, K.A. 1980. Testicular neonatal imprinting of sex dependent differences in hepatic foreign compound metabolism in the rat. Biochem. Pharmacol. 29: 3133-3137.

Forest, M.G. 1979. Plasma androgens (testosterone and 4-androstenedione) and 17-hydroxyprogesterone in the neonatal, prepubertal and peripubertal periods in the human and the rat: Differences between species. J. Steroid Biochem. 11: 543-548.

Furuhashi, N. and Fang, V.S. 1979. Sex difference in the induction of lactogenic receptors in rat liver. Acta Endocrinol. 92: 532-541.

Gold, E., Gordis, J., Tonascia, J. and Szklo, M. 1978. Increased risk of brain tumors in children exposed to barbiturates. J. Natl. Cancer Inst. 61: 1031-1034.

Gontovnick, L.S. and Bellward, G.D. 1980. Sex and age dependence of the "selective" induction of rat hepatic microsomal epoxide hydratase following trans-stilbene oxide, 1-α-acetylmethadol, or phenobarbital treatment. Biochem. Pharmacol. 29: 3245-3251.

Gorski, J. and Gannon, F. 1976. Current models of steroid hormone action: a critique. Annu. Rev. Physiol. 38: 425-450.

Guildicell, J.F. and Tillement, J.P. 1977. Influence of sex on drug kinetics in man. Clin. Pharmacokinet. 2: 157-166.

Gupta, C., Shapiro, B.H. and Yaffe, S.J. 1980. Reproductive dysfunction in male rats following prenatal exposure to phenobarbital. Pediatr. Pharmacol. 1: 55-62.

Gurtoo, H.L. and Parker, N.B. 1977. Sex-dependent regulation of benzo-(a)-pyrene and zoxazolamine metabolism in rat tissues. Drug Metab. Dispos. 5: 474-481.

Gustafsson, J.A. and Skett, P. 1978. Precocious 'feminization' of rat liver enzymes in the presence of ectopic pituitary tissue. J. Endocrinol. 76: 187-191.

Gustafsson, J.A. and Stenberg, A. 1974. Masculinization of rat liver enzyme activities following hypophysectomy. Endocrinology 95: 891-896.

Gustafsson, J.A. and Stenberg, A. 1976. On the obligatory role of the hypophysis in sexual differentiation of hepatic metabolism in rats. Proc. Natl. Acad. Sci. U.S.A. 73: 1462-1465.

Gustafsson, J.A., Pousette, A., Stenberg, A. and Wrange, O. 1975. High-affinity binding of 4-anrostene-3,17-dione in rat liver. Biochemistry 14: 3942-3948.

Gustafsson, J.A., Eneroth, P., Pousette, A., Skett, P., Sonnenschien, C., Stenberg, A. and Ahlen, A. 1977. Programming and differentiation of rat liver enzymes. J. Steroid Biochem. 8: 429-443.

Gustafsson, J.A., Eneroth, P., Hokfelt, T. and Skett, P. 1978. Central control of hepatic steroid metabolism: Effect of discrete hypothalamic lesions. Endocrinology 103: 141-151.

Hahn, E.F. and Fishman, J. 1979. Changes in rat brain opiate receptor content upon castration. Biochem. Biophys. Res. Commun. 90: 819-823.

Harada, N. and Omura, T. 1981. Selective induction of two different species of cytochrome P-450 by phenobarbital and 3-methylcholanthrene. J. Biochem. (Tokyo) 89: 237-248.

Henderson, P.T. 1971. Metabolism of drugs in rat liver during the perinatal period. Biochem. Pharmacol. 20: 1225-1232.

Kato, R. 1974. Sex-related differences in drug metabolism. Drug Metab. Rev. 3: 1-32.

Kato, R. and Gillette, J.R. 1965a. Effect of starvation on NADH--dependent enzymes in liver microsomes of male and female rats. J. Pharmacol. Exp. Ther. 150: 279-284.

Kato, R. and Gillette, J.R. 1965b. Sex differences in the effect of abnormal physiological states on the metabolism of drugs by rat liver microsomes. J. Pharmacol. Exp. Ther. 150: 285-291.

Kato, R. and Onoda, K. 1970. Studies on the regulation of the activity of drug oxidation in rat liver microsomes by androgen and estrogen. Biochem. Pharmacol. 19: 1649-1660.

Kato, R., Takahashi, A. and Omari, Y. 1968. Effect of androgen and estrogen on the hydroxylation of steroid hormones by rat liver microsomes. Endocrinol. Jpn. 16: 653-663.

Katzellenbogen, B.S. and Gorski, J. 1975. Estrogen action on synthesis of macromolecules in target cells. In Biochemical actions of hormones, Vol. 3. Edited by G. Litwach. Academic Press, New York.

Ketterer, B., Tipping, E., Meuwissen, J. and Beale, D. 1975. Ligandin. Biochem. Soc. Trans. 3: 626-630.

Ketterer, B., Tipping, E., Beale, D. and Meuwissen, J.A.T.P. 1976. Ligandin, glutathione and carcinogen binding. In Gluthathione: Metabolism and function, Vol. 6. Edited by I.M. Arias and W.B. Jakoby. Raven Press, New York. p. 243.

Kokka, N., Garcia, J.F. and Elliott, H.W. 1973. Effect of acute and chronic administration of narcotic analgesics on growth hormone and corticotropin (ACTH) secretion in rats. Prog. Brain Res. 39: 347-360.

Kramer, R.E. and Colby, H.D. 1976. Feminization of hepatic steroid and drug metabolizing enzymes by growth hormone in male rats. J. Endocrinol. 71: 449-450.

Kramer, R.E., Greiner, J.W., Canady, W.J. and Colby, H.D. 1975a. Relation of the pituitary gland to the actions of testosterone on hepatic ethylmorphine metabolism in rats. Biochem. Pharmacol. 24: 2097-2099.

Kramer, R.E., Greiner, J.W. and Colby, H.D. 1975b. Divergence of growth hormone actions on hepatic drug metabolism in the presence and absence of the pituitary gland. Life Sci. 17: 779-786.

Kramer, R.E., Greiner, J.W. and Colby, H.D. 1977. Effects of luteinizing hormone and follicle stimulating hormone on hepatic drug metabolism in gonadectomized male and female rats. Biochem. Pharmacol. 26: 66-69.

Kramer, R.E., Greiner, J.W., Rumbauch, R.C., Sweeney, T.D. and Colby, H.D. 1978. Relation of the gonadal hormones to growth hormone actions on hepatic drug metabolism in rats. J. Pharmacol. Exp. Ther. 204: 247-254.

Kramer, R.E., Greiner, J.W., Rumbaugh, R.C., Sweeney, T.D., Colby, H.D. 1979. Requirements of the pituitary gland for gonadal hormone effects on hepatic drug metabolism in rats. J. Pharmacol. Exp. Ther. 208: 19-26.

Kuntzman, R., Jacobson, M., Schneidman, K. and Conney, A.H. 1964. Similarities between oxidative drug-metabolizing enzymes and steroid hydroxylases in liver microsomes. J. Pharmacol. Exp. Ther. 146: 280-285.

LaBella, F.S. 1975. Opiate specific replacement of steroid hormones from microsomes. Life Sci. 16: 1783.

LaBella, F.S., Havlicik, V., Pinsky, C. and Leyhin, L. 1977. Opiate-like naloxone-reversible effects of androsterone sulfate in rats. Neurosci. Abst. 3: 295.

Lax, E.R., Hoff, H.G., Ghraf, R., Schroder, A. and Schnefers, H. 1974. The role of the hypophysis in the regulation of sex differences in the activities of enzymes involved in hepatic steroid hormone metabolism. Hoppe-Seyler's Z. Physiol. Chem. 355: 1325-1331.

Lu, A.Y.H. and West, S.B. 1979. Multiplicity of mammalian microsomal cytochromes P-450. Pharmacol. Rev. 31: 277-295.

Mainwaring, W.I.P., Mangane, F.R., Wilke, P.A. and Milroy, E.G.P. 1973. A review of current research on binding and mechanisms of action of androgenic steroids, notably 5α-dihydrotestosterone. Edited by B.W. O'Malley and A.R. Means. Plenum Press, New York. Adv. Exp. Med. Biol. 36: 197-231.

Marietta, M.P., Vesell, E.S., Hartman, R.D., Weisz, J. and Dvorchik, B.H. 1979. Characterization of cytochrome P-450-dependent aminopyrine N-demethylase in rat brain: Comparison with hepatic aminopyrine N-demethylation. J. Pharmacol. Exp. Ther. 208: 271-279.

Milin, B. and Roy, A.K. 1973. Androgen "receptor" in rat liver: cytosol "receptor" deficiency in pseudohermaphrodite male rats. Nat. New Biol. 242: 248-250.

Mulder, E., Van Beurden-Lamers, W.M.O., Brinkmann, A.O., Mechielsen, M.J. and Van der Molen, H.J. 1974. High affinity binding of estradiol by rat testis interstitial tissue and by several other tissues of the male rat. J. Steroid Biochem. 5: 955-959.

Opiate Symposium 1980. Fed. Proc. 39: 2531-2560.

Ota, M., Sato, N., Takahashi, S. and Ono, S. 1980. Characteristics of the binding component for testosterone and androstenedione in rat liver cytosol. Endocrinol. Jpn. 27: 321-328.

Peraino, C., Fry, F.J.M. and Stadfeldt, E. 1973a. Enhancement of spontaneous hepatic tumorigenesis in C3H mice by dietary phenobarbital. J. Natl. Cancer Inst. 51: 1349-1350.

Peraino, C., Fry, F.J.M., Stadfeldt, E. and Kisieleski, W.E. 1973b. Effects of varying the exposure to phenobarbital on its enhancement of 2-acetylaminofluorene-induced hepatic tumorigenesis in the rat. Cancer Res. 33: 2701-2708.

Pfaffenberger, C.D. and Horning, E.C. 1977. Sex differences in human urinary steroid metabolic profiles determined by gas chromatography. Anal. Biochem. 80: 329-343.

Pinsky, C., Koven, S.J. and LaBella, F.S. 1975. Evidence for role of endogenous sex steroids in morphine antinociception. Life Sci. 16: 1785.

Pitot, H.C. 1978. Drugs as promoters of carcinogenesis. In The induction of drug metabolism. Edited by R.W. Estabrook and E. Lindenlaub. Symposia Medica, Hoeschst. 14: 471-483.

Pitot, H.C. and Sirica, A.E. 1980. The stage of initiation and promotion in hepatocarcinogenesis. Biochim. Biophys. Acta 605: 191-215.

Poland, A. and Glover, E. 1979. An estimate of the maximum in vivo covalent binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin to rat liver protein, ribosomal RNA and DNA. Cancer Res. 39: 3341-3344.

Poland, A., Glover, E. and Kende, A.S. 1976. Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin by hepatic cytosol. J. Biol. Chem. 251: 4936-4946.

Posner, B.I., Kelly, P.A. and Friesen, H.G. 1974. Induction of a lactogenic receptor in rat liver: Influence of estrogen and the pituitary. Proc. Natl. Acad. Sci. U.S.A. 71: 2407-2410.

Posner, B.I., Kelly, P.A. and Friesen, H.G. 1975. Prolactin receptors in rat liver: Possible induction by prolactin. Science 188: 57-59.

Powell-Jones, W., Thompson, C., Nayfeh, S.N., Lucier, G.W. 1980. Sex differences in estrogen binding by cytosolic and nuclear components of rat liver. J. Steroid Biochem. 13: 219-229.

Prolactin Symposium 1980. Fed. Proc. 39: 2561-2598.

Quinn, G.P., Axelrod, J. and Brodie, B.B. 1958. Species, strain and sex differences in metabolism of hexobarbital, amidopyrine, antipyrine and aniline. Biochem. Pharmacol. 1: 152-159.

Roy, R.K., Milin, B.S. and McMinn, D.A. 1974. Androgen receptor in rat liver: Hormonal and developmental regulation of the cytoplasmic receptor and its correlation with the androgen-dependent synthesis of α-$_{2u}$-globulin. Biochem. Biophys. Acta 354: 213-232.

Rumbaugh, R.C. and Colby, H.D. 1980. Is growth hormone the pituitary feminizing factor mediating the actions of estradiol on hepatic drug and steroid metabolism? Endocrinology 107: 719-724.

Rumbaugh, R.C., Kramer, R.E. and Colby, H.D. 1978. Dose-dependent actions of thyroxine on hepatic drug metabolism in male and female rats. Biochem. Pharmacol. 27: 2027-2031.

Sato, N., Ota, M. and Obara, K. 1980a. Presence of binding components for testosterone in rat liver cytosol. Endocrinol. Jpn. 27: 315-319.

Sato, N., Takahashi, S. and Ota, M. 1980b. Effect of testosterone on the androgen receptor in the liver cytosol and nuclei of castrated male rats. Endocr. Res. Commun. 7: 241-252.

Skett, P. and Gustafsson, J.A. 1979. Imprinting of enzyme systems of xenobiotic and steroid metabolism. Rev. Biochem. Toxicol. 1: 27-52.

Skett, P., Eneroth, P. and Gustafsson, J.A. 1978. The development of pituitary control of hepatic steroid metabolism in the rat. Mol. Cell. Endocrinol. 10: 21-27.

Smith, G.J. and Litwack, G. 1980. Roles of ligandin and the glutathione S-transferases in binding steroid metabolites, carcinogens and other compounds. In Reviews of biochemical toxicology, Vol. 2. Edited by E. Hodgson, J.R. Bend and R.M. Philpot. Elsevier-North Holland, New York. pp. 1-47.

Sonawane, B.R., Yaffe, S.J. and Shapiro, B.H. 1979. Hepatic drug metabolism in pseudohermaphroditic rats. Drug Metab. Dispos. 7: 444-445.

Soyka, L.F. 1980. Hepatic drug metabolizing enzyme activity and tumorigenesis in mice following perinatal exposure to benzo(a)pyrene. Pediatr. Pharmacol. 1: 85-96.

Stanley, A.J., Gumbreck, L.G., Allison, J.E. and Easley, R.B. 1973. Male pseudohermaphrodism in the laboratory Norway rat. Recent Prog. Horm. Res. 29: 43-64.

Sweeney, G.C. and Cole, F.M. 1980. Effects of ethynyl estradiol on liver microsomal mixed function oxygenase activity in male rats. Lab. Invest. 42: 231-235.

Viladiu, P.C., Delgado, J., Pensky, J. and Pearson, O.H. 1975. Estrogen binding protein of rat liver. Endocr. Res. Commun. 2: 273.

POLYCHLORINATED BIPHENYLS: SPECIES AND TISSUE SPECIFICITIES OF THE INDUCTION OF MONOOXYGENASES IN RATS, MICE, RABBITS, AND HUMANS

A.P. Alvares[a], J.L. Eiseman[a], T.-H. Ueng[a] and A. Kappas[b]

ABSTRACT

The effects of polychlorinated biphenyls (PCBs) as modifiers of monooxygenases were studied in several species and tissues, as well as on two forms of cytochromes P-450, the terminal oxidase of the monoxygenase system. From data presented, it is not possible to generalize a biological effect of PCBs, namely, that it is a potent, non-specific inducer of monooxygenase activities. Although this environmental pollutant enhanced microsomal drug-metabolizing enzymes in livers of all species studied, in rabbit lung it inhibited the metabolism of ethylmorphine. At dosages used, PCBs were potent inducers of the carcinogen metabolizing enzyme, aryl hydrocarbon hydroxylase, in livers of rats, but not in mouse and rabbit liver. They proved to be potent inducers of aryl hydrocarbon hydroxylase activity in rabbit kidney; in contrast, its activity was depressed in rabbit lung. Data obtained with Aroclors 1254, 1242 and 1016 demonstrate that the inductive properties of these mixtures of PCBs may show both qualitative and quantitative differences. These studies demonstrate that the biologic impact of the widely distributed environmental pollutant, PCBs, may differ in different species and emphasize the need to carry out toxicological studies in more than one species of animal. Differential biologic effects on various organs within the same species may also be important determinants of the organ-targeted chemical toxicity.

[a]Department of Pharmacology, School of Medicine, Uniformed Services University, Bethesda, MD 20814.

[b]The Rockefeller University, New York, NY 10021.

LES POLYCHLOROBIPHENYLES: SPECIFICITE DE L'INDUCTION DES MONO-OXYGENASES RELATIVE AUX ESPECES ET AUX TISSUS, CHEZ LE RAT, LA SOURIS, LE LAPIN ET L'HOMME

A.P. Alvares[a], J.L. Eiseman[a], T.-H. Ueng[a] et A. Kappas[b]

RESUME

Les effets des polychlorobiphényles (PCB) en tant que modificateurs des monooxygénases ont été étudiés chez plusieurs espèces et tissus, ainsi que sur deux formes de cytochromes P-450, la dernière oxydase du système des monooxygénases. Les données présentées ne permettent pas d'effectuer une généralisation quant à l'effet biologique des PCB, c'est-à-dire d'affirmer qu'ils induisent de façon importante et non spécifique l'activité des monooxygénases. Même si ces pollutants environnementaux ont stimulé l'activité des enzymes microsomales responsables du métabolisme des produits pharmaceutiques dans le foie de toutes les espèces étudiées et dans le poumon de lapin, ils ont inhibé le métabolisme de l'éthylmorphine. Aux doses utilisées, les PCB ont induit de façon importante l'enzyme responsable du métabolisme des carcinogènes, l'aryl-hydrocarbure hydroxylase, dans le foie des rats mais non dans le foie des souris et des lapins. Les PCB ont induit fortement cette enzyme dans les reins de lapin; en revanche, l'activité était diminuée dans les poumons de lapin. Les données obtenues avec les Aroclors 1254, 1242 et 1016 montrent que les propriétés inductrices de ces mélanges de PCB peuvent présenter des différences à la fois qualitatives. De telles études révèlent que l'impact biologique des PCB, polluants environnementaux largement répandus, présente des différences selon l'espèce et fait ressortir la nécessité d'études toxicologiques chez plus d'une espèce. Les différences des effets biologiques selon différents organes d'une même espèce peuvent constituer des déterminants importants de la toxicité chimique pour l'organe visé.

[a]Department of Pharmacology, School of Medicine, Uniformed Services University, Bethesda, MD 20814.

[b]The Rockefeller University, New York, NY 10021.

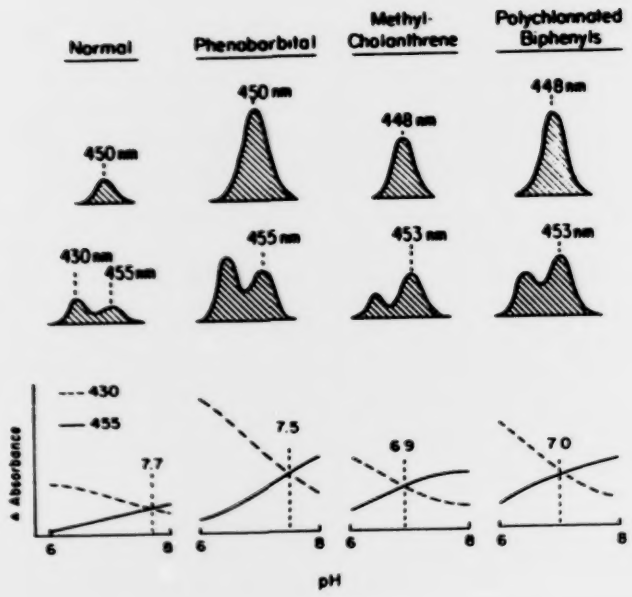
INTRODUCTION

Exposure to environmental chemicals may cause several alterations in the physiological and biochemical systems which influence absorption, distribution, metabolism and excretion of xenobiotics to which the body is exposed. Many of these substances may enhance or inhibit the activities of the monooxygenase systems, the terminal oxidase of which is a family of hemoproteins, collectively known as cytochromes P-450. Polychlorinated biphenyls (PCBs), a related group of contaminants found worldwide at low concentrations, have been employed in the past with little or no attempt to control their use or disposal. PCBs are now so pervasive that many foods, such as fish and milk - including human milk - often contain detectable concentrations of PCBs. Since 1979, the manufacture and use of PCBs have been banned in the United States, except when the chemicals are used in totally enclosed systems, such as in transformers. The disposal of waste PCBs represents a continuing source of environmental pollution and continued human exposure is likely for a long period. PCBs are extremely resistant to normal pathways of biodegradation within the ecosystem. They tend to accummulate in body fat and are resistant to biotransformation to more polar metabolites.

Commercial PCBs are a mixture of isomers manufactured in the United States under the trade name Aroclor. A major reason for the scientific interest in PCBs is because these compounds were discovered to be potent inducers of rat hepatic monooxygenases (Alvares et al. 1973). Previous investigations have shown that PCBs are inducers of steroid hydroxylases in birds (Lincer and Peakall 1970). Inducers of hepatic monooxygenases have, in general, been categorized into two groups. One group of inducers, of which phenobarbital (PB) is a prototype, enhances the metabolism of a large variety of substrates by liver cells; a second group stimulates the metabolism of only a few substrates. Polycyclic hydrocarbon carcinogens, such as benzo(a)pyrene and 3-methylcholanthrene (3-MC) constitute the second group of compounds. More recent studies have shown that the environmental pollutant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces two distinct forms of cytochrome P-450 (Johnson et al. 1979). The various forms of hepatic cytochromes induced by drugs and environmental chemicals have been characterized by spectral, catalytic, gel-electrophoretic, immunochemical, peptide mapping and other techniques.

PROPERTIES OF HEPATIC MONOOXYGENASES FROM RATS TREATED WITH AROCLOR 1254

Studies carried out to date indicate that the PCBs mixture, Aroclor 1254, possesses the inducing properties of both the PB and 3-MC classes of inducers in rat hepatic endoplasmic reticulum. A summary of evidence based on spectral and catalytic properties, using liver microsomes from rats pretreated with the various inducers, is depicted in Figure 1. The three inducers increased the cytochrome P-450 levels;



	Normal	Phenobarbital	Methyl-Cholanthrene	Polychlorinated Biphenyls
Ethylmorphine N-demethylase (μ mol HCHO/mg protein/hr)	0.43	1.59	0.47	1.15
Benzo[a]pyrene Hydroxylase (n mol OHBP/mg protein/hr)	7.50	20.09	58.52	47.94

Figure 1. Differences in spectral and catalytic properties of liver microsomes from rats pretreated with PB, 3-MC and Aroclor 1254 (PCB). Spectra depicted are the CO-difference spectra, ethyl isocyanide-difference spectra at pH 7.4, and pH-dependent intercepts of the interconvertible forms of reduced hemoprotein combined with ethyl isocyanide. Ethylmorphine N-demethylase was determined on microsomal suspensions and benzo(a)pyrene hydroxylase on 9000 x g supernatants. Dosages: PB, 75 mg/kg/d for 4 d; 3-MC, 25 mg/kg/d for 4 d; Aroclor 1254, 25 mg/kg/d for 6 d. Compounds were administered intraperitoneally and rats were killed 24 h after the last injection.

however, the CO-difference spectra showed absorption maxima at 448, 448, and 450 nm with the PCB mixture, Aroclor 1254, 3-MC, and PB, respectively. If ethyl isocyanide is used instead of CO as a ligand for the reduced hemoprotein, Soret peaks at 430 and 455 nm are observed (Imai and Sato 1966) and these peak heights are pH dependent. Using microsomes from untreated rats, Imai and Sato (1966) plotted the peak heights at various pHs and found that the peak heights intercepted at pH 7.4. Sladek and Mannering (1966) confirmed that the intercepts obtained with microsomes from untreated and PB-treated rats intercepted at pH 7.5-7.7; whereas with microsomes from 3-MC-treated rats, the intercept occurred at pH 6.9 (Fig. 1). As can be seen in Figure 1, microsomes from Aroclor 1254-treated rats gave an intercept at pH 7.0, which was intermediate between PB- and 3-MC-treated rats. PCBs also caused a 6-fold induction of benzo(a)pyrene hydroxylase activity, whereas 3-MC and PB caused 8- and 3-fold induction of the hydroxylase activity, respectively. As expected, 3-MC had no effect on the activity of ethylmorphine N-demethylase (Alvares et al. 1973). In this respect, PCBs produced effects similar to those observed following PB treatment; namely, they caused a marked enhancement of ethylmorphine N-demethylase activity. These data and other studies carried out in our laboratory (Alvares and Siekevitz 1973; Alvares and Kappas 1977) provided conclusive evidence that in the male rat, Aroclor 1254 shares the inducing properties of the PB and 3-MC classes of inducers and induces both cytochromes P-450 and P-448 and associated enzymic activities. Using microsomes from Aroclor 1254-treated rats, Ryan et al. (1977,1979) have obtained highly purified cytochromes P-450 and using various other criteria, including gel electrophoretic and immunochemical characteristics, they have shown that the forms of cytochrome P-450 were identical to the corresponding hemoproteins from PB- or 3-MC-treated rats.

COMPARATIVE EFFECTS OF AROCLORS 1242 and 1016 ON RAT HEPATIC MONOOXYGENASES

Although various investigators have used predominantly Aroclor 1254 to study the induction of various forms of cytochrome P-450, our earlier studies (Bickers et al. 1972) had shown that the two PCB mixtures, Aroclors 1254 and 1016, differed in their inductive effects. Aroclor 1254, for example, enhanced ethylmorphine N-demethylase activity 3-fold, while the maximum increase produced by Aroclor 1016 was only 40%. In those studies, no attempt was made to determine if Aroclor 1016 induced the synthesis of cytochrome P-448 and associated benzo(a)pyrene hydroxylase activity. In the last several years, Aroclor 1016 has been the major PCB mixture marketed in the United States. Aroclor 1254 and 1016 differ in both the congener composition as well as in the degree of chlorination (Ecobichon and Comeau 1974). Aroclor 1254 contains 54% chlorine and is composed primarily of the higher penta- and hexachlorine containing biphenyl isomers. Aroclor 1016 contains primarily di-, tri- and tetrachlorobiphenyl isomers.

Another commercial available PCBs mixture is Aroclor 1242. This PCBs mixture has a similar chlorine content (42%) as Aroclor 1016 (41%). However, Aroclor 1242 contains approximately 10% of pentachlorobiphenyl congeners, whereas Aroclor 1016 contains approximately 1% of pentachlorobiphenyls (Ecobichon and Comeau 1974). Because of reports that the lower chlorine containing isomers are metabolized more readily, it was of interest to compare the inducing properties of Aroclor 1242 and 1016, with respect to their abilities to induce cytochrome P-450-dependent ethylmorphine N-demethylase and cytochrome P-448-dependent benzo(a)pyrene hydroxylase activities, and to determine their effects on the spectral properties of the induced cytochrome. The data are presented in Table 1.

In these experiments, Aroclor 1242 treatment of rats resulted in spectral changes in hepatic microsomal cytochrome P-450 similar to those elicited by Aroclor 1254. Both Aroclor 1242 and 1016 caused significant increases in cytochrome P-450 contents; however, only Aroclor 1242 caused a shift in absorbance maximum to a lower wavelength in the Soret region. Also, like Aroclor 1254 (Fig. 1), Aroclor 1242 caused a marked increase in the 455 nm peak height. Thus, the increase in the 455:430 nm ratio of the ethyl isocyanide-induced difference spectrum observed with Aroclor 1242 was much greater than that elicited by Aroclor 1016. These data, plus the minimal induction of benzo(a)pyrene hydroxylase (Table 1) demonstrate that Aroclor 1016 induces primarily cytochrome P-450 and associated N-demethylase activity; and that this PCB mixture did not significantly induce the carcinogen-metabolizing enzyme, cytochrome P-448.

EFFECTS OF PCBs ON DRUG METABOLISM IN MAN

To determine if the above findings in rats extend to man, we studied the effects of exposure to PCBs in workers in a capacitor-manufacturing plant. Five workers were selected who had been exposed to PCBs for 4-16 years. For at least 2 years prior to our study, the workers had been exposed to Aroclor 1016. Five normal subjects, who had no previous known exposure to PCBs, were selected as control subjects and were matched as closely as possible with respect to age, sex, smoking and drinking habits. None of the PCB exposed workers studied showed any clinical manifestations of toxicity, such as chloracne, eye discharge, skin pigmentation, or gastrointestinal or neurologic dysfunction (Kuratsune et al. 1972).

It is feasible to assess the functional capacity of the cytochrome P-450 system in man by following the plasma elimination rate ($t_{\frac{1}{2}}$) of antipyrine, a drug that is completely absorbed when given orally and is completely metabolized by the liver microsomal enzyme system (Brodie and Axelrod 1950). The kinetics of antipyrine elimination were determined in the 5 workers occupationally exposed to Aroclor 1016 and the results are presented in Figure 2. The mean plasma $t_{\frac{1}{2}}$ of 10.8 h in the subjects exposed to PCBs was on an average 31% shorter ($p < 0.005$)

Table 1. Effects of Aroclors 1242 and 1016 on hepatic monooxygenases.

Assay	Control	Aroclor 1242	Aroclor 1016
CYTOCHROME P-450, nmol/mg protein	0.62 ± 0.03	1.72* ± 0.08	1.46* ± 0.01
ETHYLMORPHINE N-DEMETHYLASE[α]	0.16 ± 0.01	0.67* ± 0.03	0.59* ± 0.08
BENZO(A)PYRENE HYDROXYLASE[β]	13.89 ± 0.79	140.96* ± 11.83	21.58* ± 2.25
455/430 nm PEAKS	0.59 ± 0.02	1.24* ± 0.03	0.81* ± 0.03
CO-DIFFERENCE SPECTRUM absorbance max., nm	450	449	450

Male Sprague Dawley rats were administered, intraperitoneally, Aroclor 1242 or 1016, 50 mg/kg/d, for 4 d. Asterisk represents value significantly different from the respective control value ($p < 0.05$).

[α] Units are μmol formaldehyde produced/mg protein/h.

[β] Units are nmol hydroxy benzo(a)pyrene produced/mg protein/h.

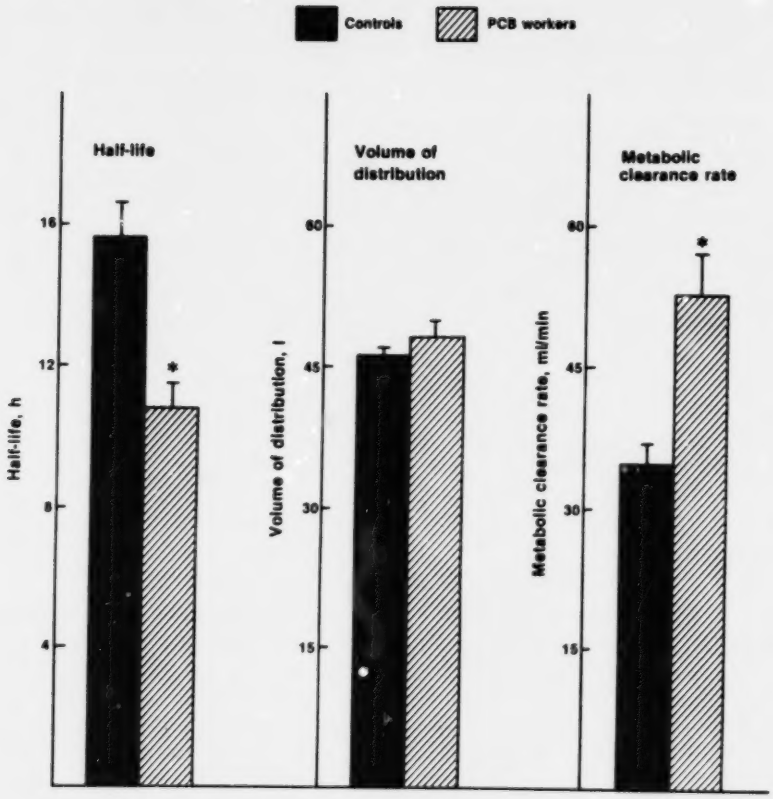


Figure 2. Effects of human exposure to PCBs on the metabolism of antipyrine. Controls and workers occupationally exposed to PCB-containing Aroclor for at least 4 years were administered antipyrine, 18 mg/kg, orally, and plasma concentrations of the drug were determined at various time intervals following the administration of the drug. Each bar represents mean ± S.E. for 5 subjects. Asterisk represents value significantly different ($p < 0.01$) from the respective control value. Data taken from Alvares et al. (1977).

than the mean $t_{\frac{1}{2}}$ of 15.6 h in the control subjects. There was no significant difference in the mean apparent volumes of distribution of the drug between the two groups studied. There was, however, a 52% more rapid ($p < 0.01$) metabolic clearance rate of the drug in workers handling Aroclor 1016 than in the control subjects (Fig. 2). The change in clearance rate without a change in the volume of distribution strongly suggests that the rate of metabolism of antipyrine was accelerated in the exposed workers. These data may have considerable importance in relation to the pharmacologic effects of other drugs that are taken by individuals exposed to halogenated hydrocarbons.

SPECIES DIFFERENCES IN THE INDUCTION OF HEPATIC MONOOXYGENASES BY AROCLOR 1254

The metabolism of many drugs and environmental chemicals is known to vary with species. The available evidence indicates that the presence of different cytochrome P-450 isozymes in tissues from various sources is primarily responsible for the observed differences in metabolism. Although cytochrome P-450 from different animals treated with the same inducer may show identical absorption maxima, purified preparations have provided evidence that they are indeed different proteins. Cytochrome P-448 purified from 3-MC-treated rats was approximately 20-fold more effective than cytochrome P-448 purified from 3-MC treated rabbits in catalyzing the hydroxylation of benzo(a)pyrene (Kawalek et al. 1975). Studies by Thomas et al. (1972) and Gielen et al. (1972) have shown that 3-MC stimulates benzo(a)pyrene hydroxylase activity in "responsive" C57BL/6 inbred strain of mice, but not in the "nonresponsive" DBA/2 inbred strain. In an extensive series of studies with genetically inbred mice, Nebert and associates have since shown that the murine Ah locus regulates the 3-MC-dependent induction of numerous drug metabolizing enzyme activities in the liver (Nebert and Jensen 1979). However, ethylmorphine N-demethylase does not appear to be associated with the Ah locus (Thorgeirsson and Nebert 1977).

Since our studies demonstrated that in rats, Aroclor 1254 posessed both the PB and 3-MC type inductive properties, it was of interest to determine if this PCB mixture exhibited similar inductive properties in the random-bred Swiss strain and in the inbred strains C57BL/6J and DBA/2J mice. The Swiss strain was obtained from the Rockefeller University and the inbred strains from Jackson Laboratories, Bar Harbor, Maine. The random bred strain was used in these studies, because the human population is certainly more randomly bred. Hence, the Swiss would be an appropriate strain to study, and to compare the data with those of the inbred mouse populations. A comparison of the effects of PB, 3-MC, and the PCBs mixture, Aroclor 1254, on the induction of cytochrome P-450, ethylmorphine N-demethylase and benzo(a)pyrene hydroxylase activities in liver microsomes is shown in Figures 3-5. PB significantly induced cytochrome P-450 and associated enzymic activities in all three strains studied. As expected, 3-MC

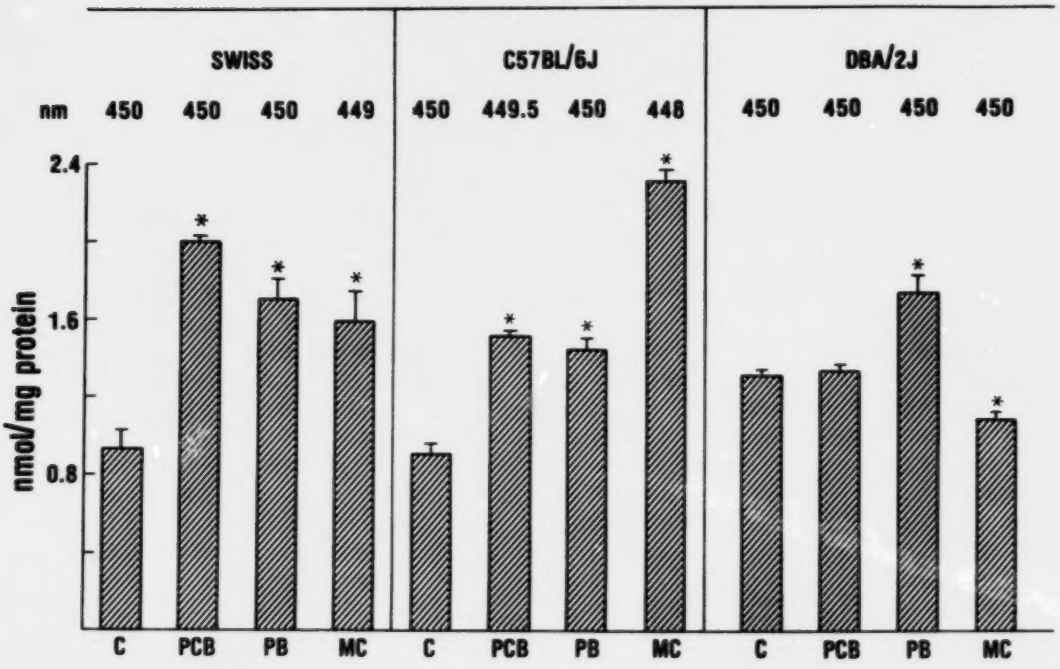


Figure 3. Cytochrome P-450 contents of liver microsomes from random bred Swiss mice and from inbred C57BL/6J and DBA/2J mice. Wavelength values at top of the respective bars represent absorbance maximum in nm of the CO-difference spectrum of the reduced microsomes. Adult male mice were administered intraperitoneally: PB, 75 mg/kg/d for 4 d; 3-MC, 50 mg/kg/d for 4 d; Aroclor 1254 (PCB), 50 mg/kg/d for 4 d. Each bar represents mean ± S.E. for 8 mice; livers from two mice were pooled for each assay. Asterisk represents value significantly different from the respective control (C) value ($p < 0.05$).

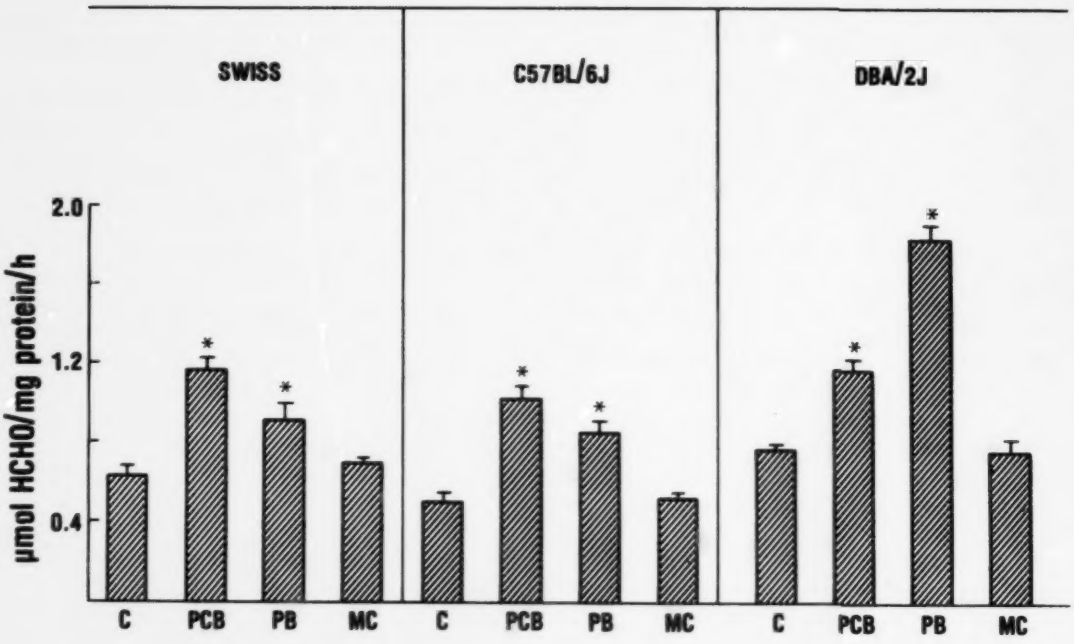


Figure 4. Ethylmorphine N-demethylase activities activities of liver microsomes from random bred Swiss mice and from inbred C57BL/6J and DBA/2J mice. See legend to Figure 3 for abbreviations and dosages. Asterisk represents value significantly different from the respective control value ($p < 0.05$).

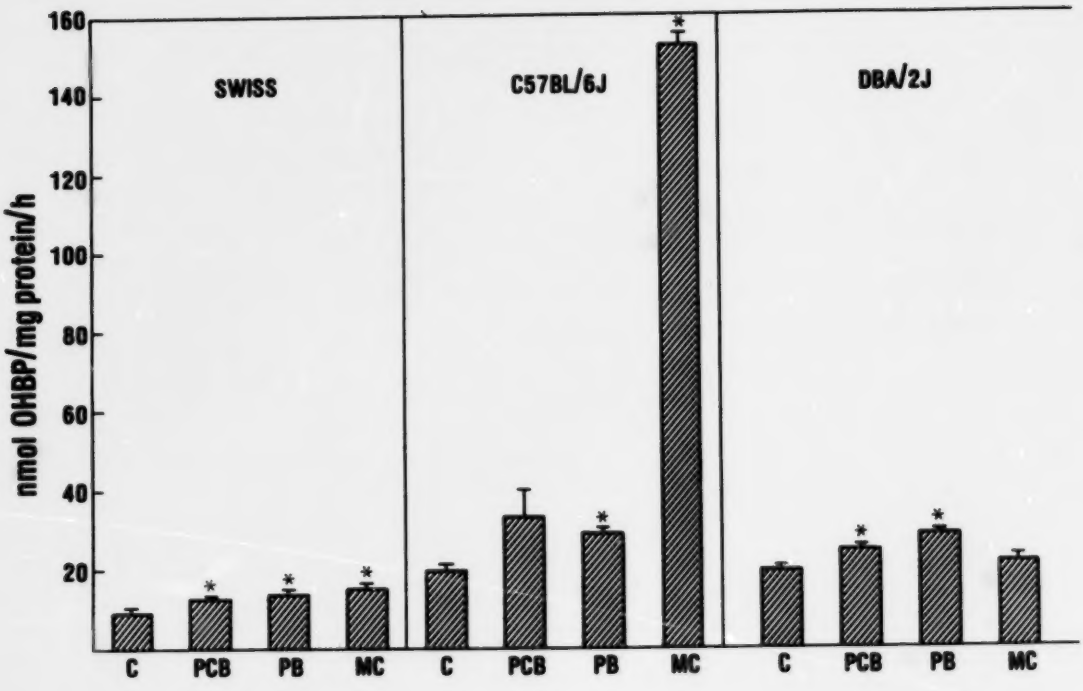


Figure 5. Benzo(a)pyrene hydroxylase activities of liver microsomes from random bred Swiss mice and from inbred C57BL/6J and DBA/2J mice. See legend to Figure 3 for abbreviations and dosages. Asterisk represents value significantly different from the respective control value ($p < 0.05$).

caused no significant induction of N-demethylase activity (Fig. 4) in any of the strains studied and a marked 8-fold induction of benzo(a)pyrene hydroxylase activity in the "responsive" C57BL/6J mice (Fig. 5). The polycyclic hydrocarbon treatment resulted in absorbance maxima of 450, 449, and 448 nm in the CO-difference spectrum of liver microsomes from in DBA/2J, Swiss, and C57BL/6J mice, respectively. Aroclor 1254-treatment caused minimal changes in benzo(a)pyrene hydroxylase activities in all three strains studied. In the random bred Swiss strain, in the "responsive" strain, and to a lesser extent in the "nonresponsive" strains of mice tested, the inductive properties of Aroclor 1254 were similar to those elicited by PB treatment, rather than by 3-MC-treatment (Figs. 3-5). Thus, a comparison of the biological properties of the PCBs in rats and mice demonstrate species differences in the mode of induction of hepatic monooxygenases.

Although under our experimental conditions, PCBs did not induce benzo(a)pyrene hydroxylase activities in either the "responsive" or the "nonresponsive" strains of mice, Robinson et al. (1975) using ten times the dose of Aroclor 1254 as in the present studies did observe enhancement of benzo(a)pyrene hydroxylase activity in both the C57BL/6N and DBA/2N strains of mice obtained from the National Institutes of Health, Bethesda, MD. The latter effects may be due to the inducing properties of polychlorinated dibenzofurans, minor impurities present in commercial PCBs (Kawano and Hiraga 1978). Commercial preparations of PCBs are contaminated with small amounts of polychlorinated dibenzofurans 1.5-28 $mg.m^{-3}$ (1 to 20 ppm). Such a trace contamination of the dibenzofurans may be responsible for the induction of benzo(a)pyrene hydroxylase activity observed when very large doses of PCBs are administered to "responsive" and "nonresponsive" mice. Kawano and Hiraga (1978) have shown that dibenzofurans are 3-MC type inducers of rat hepatic monooxygenases.

TISSUE SPECIFICITIES IN THE INDUCIBILITY OF MONOOXYGENASES BY AROCLOR 1254

Recent studies (Alvares et al. 1980) have shown that in rabbit liver, Aroclor 1254-treatment, like 3-MC, induced cytochrome P-448 and N-demethylase activity but caused no significant induction of aryl hydrocarbon hydroxylase activity. These data are similar to those previously reported using 3-MC as an inducer of rabbit liver monooxygenases (Atlas et al. 1975). In the latter studies, treatment of adult rabbits resulted in hepatic cytochrome P-448 induction, with no increase in benzo(a)pyrene hydroxylation.

Since the lung may be a portal of entry and the kidney a portal of excretion of xenobiotics, it was of interest to determine the effects of PCBs on these two extrahepatic tissues (Alvares et al. 1982). The kidney has a relatively high cytochrome P-450 content, although it is substantially lower than that of liver. Administration of Aroclor 1254 to New Zealand white rabbits resulted in differential effects on

the monooxygenase systems in the liver, kidney, and lung (Table 2). In the liver, Aroclor 1254 caused a significant induction of cytochrome P-450 and ethylmorphine N-demethylase activity, but no significant induction of benzo(a)pyrene hydroxylase activity. In contrast to the liver, PCB treatment caused a decrease in pulmonary cytochrome P-450 content and ethylmorphine N-demethylase and benzo(a)pyrene hydroxylase activities. In marked contrast to the other tissues studied, PCB pretreatment caused a 5-fold increase in renal benzo(a)pyrene hydroxylase activity. PCBs also caused a 2- to 3-fold increase in renal cytochrome P-450 and N-demethylase activity (Table 2). Although the PCBs caused a shift in the absorbance maximum of the CO-difference spectrum of rabbit liver microsomes to 448 nm, no such shift in the Soret region was observed with kidney microsomes. These data obtained with rabbits, differ with those obtained with rats pretreated with Aroclor 1254. Previous studies have shown that the PCBs mixture markedly enhanced benzo(a)pyrene hydroxylase activities in liver, kidney, lung, placenta, adrenal and testis of rats (Alvares and Kappas 1977).

The above data demonstrate that in the three rabbit tissues studied, the inductive effects of PCBs are tissue specific. The monooxygenases are capable of metabolizing a wide variety of endogenous and exogenous chemicals. Although metabolism by these enzymes in many cases produce compounds with reduced biological activity, in some specific instances, as with polycyclic aromatic hydrocarbons, metabolic transformation may result in more active or toxic metabolites. Differences in tissue-specific metabolism, due to differential induction of monooxygenases by environmental chemicals such as PCBs, may be important determinants of the local concentrations of drugs and environmental toxicants and their metabolites. Such differences in concentrations of biologically active compounds may play an important role in the pathophysiology of chemical toxicity.

DIFFERENTIAL EFFECTS OF AROCLOR 1254 ON RABBIT PULMONARY CYTOCHROMES P-450

The existence of at least two forms of rabbit pulmonary cytochrome (Wolf *et al.* 1978; Guengerich 1977), termed cytochromes P-450_I and P-450_{II}, has been demonstrated and the isozymes appear to be present in approximately equal amounts in lungs of untreated rabbits. These two forms in untreated rabbit lungs differ in their catalytic and biochemical properties (Serabjit-Singh *et al.* 1979). Since Aroclor 1254 inhibited pulmonary enzymic activities, we have utilized the purification procedures of Philpot and associates (Wolf *et al.* 1978; Arinc and Philpot 1976) to determine if Aroclor 1254 inhibited cytochromes P-450_I and P-450_{II} in lungs from rabbits pretreated with the PCBs mixture. The hemoproteins isolated from lungs of Aroclor 1254-treated rabbits differed in their spectral properties (Fig. 6). The absorbance maxima of the CO-difference spectra of cytochromes P-450_I and P-450_{II} occurred at 452 and 450 nm, respectively.

Table 2. Effect of Aroclor 1254 on rabbit tissue monooxygenase activities.

Assay	Liver		Lung		Kidney	
	Control	Treated	Control	Treated	Control	Treated
CYTOCHROME P-450, nmol/mg protein	0.77 ± 0.08	1.21* ± 0.05	0.26 ± 0.03	0.14* ± 0.01	0.18 ± 0.02	0.48* ± 0.07
ETHYLMORPHINE N-DEMETHYLASE[α]	77.2 ± 7.2	135.6* ± 6.2	38.6 ± 4.1	11.4* ± 2.2	12.2 ± 2.9	27.5* ± 3.9
BENZO(a)PYRENE HYDROXYLASE[β]	16.8 ± 1.7	22.2 ± 1.5	1.26 ± 0.13	0.87* ± 0.06	0.61 ± 0.08	3.38* ± 0.85

Male New Zealand rabbits were administered Aroclor 1254 intraperitoneally, 100 mg/kg on days 1 and 4, and killed on day 7. Each value represents mean ± S.E. for tissue obtained from 4 rabbits. Asterisks represent values significantly different from the control group ($p < 0.05$).

[α, β] Same as Table 1.

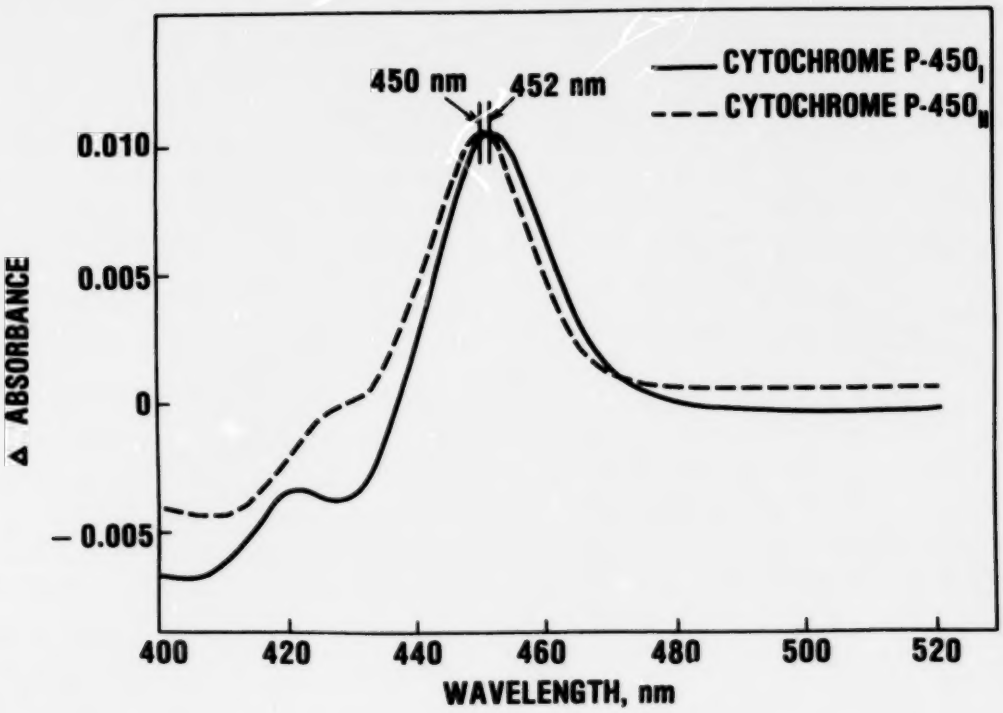


Figure 6. CO-difference spectra of pulmonary cytochromes P-450$_I$ and P-450$_{II}$ from Aroclor 1254-treated rabbits. Male New Zealand white rabbits were administered intraperitoneally with Aroclor 1254, 100 mg/kg, on days 1 and 4, and killed on day 7. Microsomes were solubilized and chromatographed on DE-52 columns as described by Ueng and Alvares (1981). DE-52 column fractions associated with cytochromes P-450$_I$ and P-450$_{II}$ were pooled and concentrated. Protein concentrations for the spectral determinations of cytochromes P-450$_I$ and P-450$_{II}$ were 0.16 mg and 0.10 mg per mL buffer, respectively.

Prior to determining the spectral properties of the pulmonary cytochromes, microsomes were prepared from lungs of untreated and Aroclor 1254-treated rabbits. Microsomal preparations, containing approximately equal amounts of protein, were solubilized using sodium cholate, dialyzed overnight and chromatographed on DE-52 columns, as described previously (Ueng and Alvares 1981). The column elution profiles from untreated and Aroclor 1254-treated rabbits are shown in Figure 7. There was a distinct separation of cytochromes P-450$_I$ and P-450$_{II}$. In agreement with the observations of Wolf et al. (1978), the elution profile shows that cytochromes P-450$_I$ and P-450$_{II}$ are present in approximately equal amounts in the lung microsomal preparations obtained from untreated rabbits. In contrast, the elution profile of solubilized microsomes from rabbits treated with Aroclor 1254 showed no significant quantitative change in the elution of cytochrome P-450$_{II}$, but did show a marked decrease in cytochrome P-450$_I$ content (Fig. 7). The selective loss of one hemoprotein with minimal loss of the other may explain the differences in the inhibitory effects of Aroclor 1254 on ethylmorphine N-demethylase and benzo(a)pyrene hydroxylase activities (Table 2). A greater decrease in N-demethylase activity than in hydroxylase activity was observed with pulmonary microsomes from Aroclor 1254-treated rabbits. It should be noted that purified P-450 preparations generally possess different but overlapping substrate specificities when reconstituted in vitro.

SUMMARY

The studies reported here demonstrate that commercial PCB mixtures which humans may be exposed to occupationally or as environmental pollutants, cannot always be considered as potent, nonspecific inducers of liver microsomal enzymes. In general, they appeared more readily to enhance the activity of the drug-metabolizing enzymes than the carcinogen-metabolizing enzyme, aryl hydrocarbon (benzo(a)pyrene) hydroxylase. Hence, it is to be expected that such individuals may respond differently to drugs which they may ingest, particularly drugs that have a low therapeutic index.

Inducibility of monooxygenases may be determined by the composition and tissue distribution of impurities present in environmental toxicants. Aroclor 1242, for example, although possessing the same degree of chlorination as Aroclor 1016, was a more potent and relatively less specific inducer of monooxygenases than Aroclor 1016. Aroclor 1016 has a larger proportion of lower chlorine-containing isomers of chlorinated biphenyls than Aroclor 1242, and induced primarily the drug-metabolizing enzyme system.

The data presented also demonstrate that the biological effects of environmental toxicants may exhibit species specificity. The carcinogen-metabolizing enzyme, aryl hydrocarbon hydroxylase, for example, was markedly induced in rat liver and rabbit kidney. However,

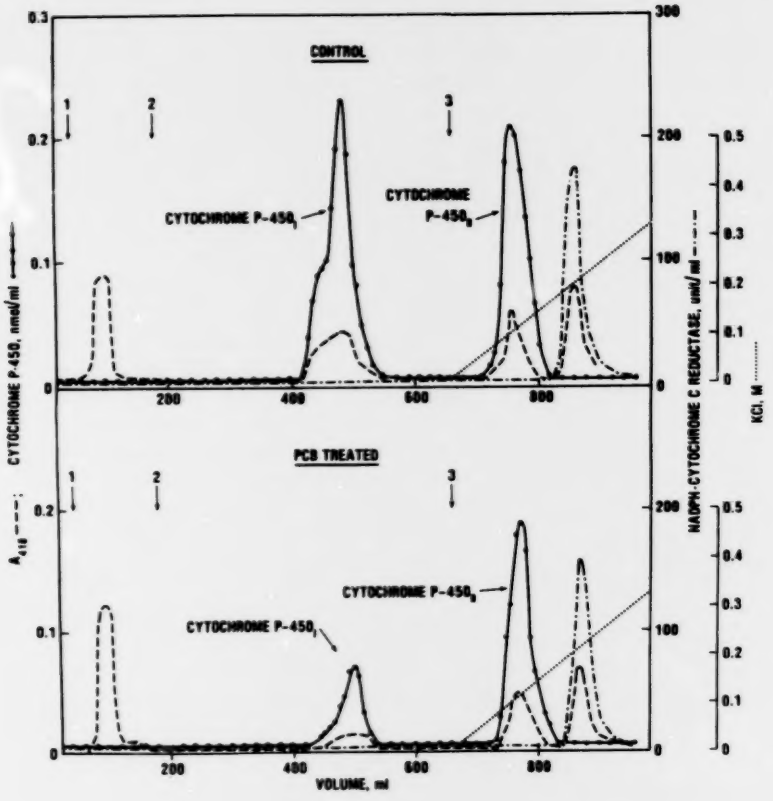


Figure 7. Column chromatography of solubilized lung microsomes from untreated and Aroclor 1254-treated rabbits. Approximately 50 mg of microsomal protein were applied to Whatman DE-52 columns, previously equilibrated with 10 mM phosphate buffer, pH 7.7, containing 20% glycerol (v/v), 0.1 mM EDTA, 0.1 mM dithiothreitol and 0.1% sodium cholate. Both columns were run simultaneously. Arrows represent changes in elution buffers: 1. Equilibration buffer; 2. Equilibration buffer containing 0.2% Emulgen 911; 3. Start of 0-0.5 M KCl gradient. A_{418} was used as a measure of hemoprotein eluting from the column. See legend to Figure 6 for dosage and treatment of rabbits. Data taken from Ueng and Alvares (1981).

in the rabbit, the activity of the liver enzyme was not affected, whereas in the lung, it was markedly inhibited.

In addition, the data presented also provide evidence that PCBs may exert their biological effects only on certain forms of cytochrome P-450. For example, Aroclor 1254 inhibited cytochrome P-450$_I$, but not cytochrome P-450$_{II}$, in rabbit lung. The differential effects of drugs, such as barbiturates, and environmentally-derived chemicals, such as polycyclic aromatic hydrocarbons and 2,3,7,8-tetrachloro-p-dioxin (TCDD), on the induction of specific forms of cytochrome P-450 have been well-established (Lu and West 1980).

The monooxygenases, in addition to oxidizing foreign compounds, can also metabolize several endogenous substrates, such as steroid hormones and fatty acids. Treatment of rats with an inducer such as PB enhances the hydroxylation of androgens, estrogens, glucocorticoids, and progestational steroids by liver microsomal enzymes. This effect is paralleled in vivo by enhanced metabolism and decreased action of steroids, such as estradiol, estrone and progesterone. It would be of considerable importance to determine if individuals occupationally exposed to enzyme inducers metabolize steroid hormones at a faster rate than the non-exposed general population.

Most investigations to date have involved studies on the biological and toxicological effects of a single agent and chemical interactions has been largely neglected. Both ethanol and polycyclic aromatic hydrocarbons present in cigarette smoke induce monooxygenase activities. Consequently, the response of an individual exposed to a chemical agent may be different if the individual also ingests alcohol or smoke tobacco in large quantities.

The propensity for such chemical-chemical interactions to produce a variety of toxic effects, including disorders of the nervous, hematopoietic, and reproductive systems, or enhancement of latent neoplastic changes, via promotion or cocarcinogensis needs to be determined. Such interactions may result in additive, synergistic or antagonistic toxic effects. Finally, it should be emphasized that induction or inhibition of the monooxygenase systems in hepatic and nonhepatic tissues may not necessarily lead to increased detoxification and excretion, but might result in enhanced formation of toxic or carcinogenic intermediates. For example, recent studies have shown that drug toxicity may be related to the formation of such intermediates which bind covalently to intracellular macromolecules, resulting in tissue damage.

ACKNOWLEDGEMENTS

The authors thank Mr. Mark O. Chapman for his secretarial help in the preparation of this manuscript. This study was supported in part by USPHS grant ES-01055.

REFERENCES

Alvares, A.P. and Kappas, A. 1977. Heterogeneity of cytochrome P-450s induced by polychlorinated biphenyls. J. Biol. Chem. 252: 6373-6378.

Alvares, A.P. and Siekevitz, P. 1973. Gel electrophoresis of partially purified cytochromes P-450 from liver microsomes of variously treated rats. Biochem. Biophys. Res. Commun. 54: 923-929.

Alvares, A.P., Bickers, D.R. and Kappas, A. 1973. Polychlorinated biphenyls: a new type of inducer of cytochrome P-448 in the liver. Proc. Natl. Acad. Sci. U.S.A 70: 1321-1325.

Alvares, A.P., Fischbein, A., Anderson, K.E. and Kappas, A. 1977. Alterations in drug metabolism in workers exposed to polychlorinated biphenyls. Clin. Pharmacol. Ther. 22: 140-146.

Alvares, A.P., Eiseman, J.L. and Yang, S.K. 1980. Inducibility of the metabolism of benzo(a)pyrene by polychlorinated biphenyls (PCBs) in liver and lung microsomal preparations from rats and rabbits. In Microsomes, drug oxidations, and chemical carcinogenesis. Edited by M.J. Coon, A.H. Conney, R.W. Estabrook, H.V Gelboin, J.R. Gillette and P.J. O'Brien. Academic Press, New York. pp. 1207-1210.

Alvares, A.P., Ueng, T.-H. and Eiseman, J.L. 1982. Polychlorinated biphenyls (PCBs): inducible monooxygenases in rabbits and mice: Species and organ specificities. Life Sci. 30: 747-751.

Arinc, E. and Philpot, R.M. 1976. Preparation and properties of partially purified pulmonary cytochrome P-450 from rabbits. J. Biol. Chem. 251: 3213-3220.

Atlas, S.A., Thorgeirsson, S.S., Boobis, A.R., Kumaki, K. and Nebert, D.W. 1975. Differential induction of murine Ah locus-associated monooxygenase activities in rabbit liver and kidney. Biochem. Pharmacol. 24: 2111-2116.

Bickers, D.R., Harber, L.C., Kappas, A. and Alvares, A.P. 1972. Polychlorinated biphenyls: comparative effects of high and low chlorine containing Aroclors on hepatic mixed function oxidase. Res. Commun. Chem. Pathol. Pharmacol. 3: 505-512.

Brodie, B.B. and Axelrod, J. 1950. The fate of antipyrine in man. J. Pharmacol. Exp. Ther. 98: 97-104.

Ecobichon, D.J. and Comeau, A.M. 1974. Comparative effects of commercial Aroclors on rat liver enzyme activities. Chem. Biol. Interact. 9: 341-350.

Gielen, J.E., Goujon, F.M. and Nebert, D.W. 1972. Genetic regulation of aryl hydrocarbon hydroxylase induction. II. Simple Mendelian expression in mouse tissues in vivo. J. Biol. Chem. 247: 1125-1137.

Guengerich, F.P. 1977. Preparation and properties of highly-purified cytochrome P-450 and reductase from pulmonary microsomes of untreated rabbits. Mol. Pharmacol. 13: 911-923.

Imai, Y. and Sato, R. 1966. Evidence for two forms of P-450 hemoprotein in microsomal membranes. Biochem. Biophys. Res. Commun. 23: 5-11.

Johnson, E.F., Zounes, M.C. and Muller-Eberhard, U. 1979. Characterization of three forms of rabbit microsomal cytochrome P-450 by peptide mapping utilizing limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. Arch. Biochem. Biophys. 192: 282-289.

Kawalek, J.C., Levin, W., Ryan, D., Thomas, P.E., and Lu, A.Y.H. 1975. Purification of liver microsomal cytochrome P-448 from 3-methylcholanthrene-treated rabbits. Mol. Pharmacol. 11: 874-878.

Kawano, S. and Hiraga, K. 1978. Polychlorinated dibenzofurans - potent inducers of rat hepatic drug-metabolizing enzymes. Jpn. J. Pharmacol. 28: 305-315.

Kuratsune, M., Yoshimura, T., Matsuzaka, J. and Yamaguchi, A. 1972. Epidemiologic study on Yusho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. Environ. Health Perspect. 1: 119-128.

Lincer, J.L. and Peakall, D.B. 1970. Metabolic effects of polychlorinated biphenyls in the American kestrel. Nature 228: 783-784.

Lu, A.Y.H. and West, S.B. 1980. Multiplicity of mammalian cytochromes P-450. Pharmacol. Rev. 31: 277-295.

Nebert, D.W. and Jensen, N.M. 1979. The Ah locus: Genetic regulation of the metabolism of carcinogens, drugs, and other environmental chemicals by cytochrome P-450 mediated monooxygenase. CRC Crit. Rev. Biochem. 6: 401-437.

Robinson, J.R., Felton, J.S., Levitt, R.C., Thorgeirsson, S.S. and Nebert, D.W. 1975. Relationship between "aromatic hydrocarbon responsiveness" and the survival times in mice treated with various drug and environmental compounds. Mol. Pharmacol. 11: 850-865.

Ryan, D.E., Thomas, P.E. and Levin, W. 1977. Properties of purified liver microsomal cytochrome P-450 from rats treated with the polychlorinated biphenyl mixture Aroclor 1254. Mol. Pharmacol. 13: 521-532.

Ryan, D.E., Thomas, P.E., Korzeniowski, D. and Levin, W. 1979. Separation and characterization of highly purified forms of liver microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls, phenobarbital, and 3-methylcholanthrene. J. Biol. Chem. 254: 1365-1374.

Serabjit-Singh, C.J., Wolf, C.R. and Philpot, R.M. 1979. The rabbit pulmonary monooxygenase system. Immunological and biochemical characterization of enzyme components. J. Biol. Chem. 254: 9901-9907.

Sladek, N.E. and Mannering, G.J. 1966. Evidence for a new P-450 hemoprotein in microsomes from methylcholanthrene-treated rats. Biochem. Biophys. Res. Commun. 24: 668-674.

Thomas, P.E., Kouri, R.E. and Hutton, J.J. 1972. The genetics of aryl hydrocarbon hydroxylase induction in mice: A single gene difference between C57BL/6J and DBA/2J. Biochem. Genet. 6: 157-168.

Thorgeirsson, S.S. and Nebert, D.W. 1977. The Ah locus and the metabolism of chemical carcinogens and other foreign compounds. Adv. Cancer Res. 25: 149-193.

Ueng, T.-H. and Alvares, A.P. 1981. Selective loss of pulmonary cytochrome P-450$_I$ in rabbits pretreated with polychlorinated biphenyls. J. Biol. Chem. 256: 7536-7542.

Wolf, C.R., Szutowski, M.M., Ball, L.M. and Philpot, R.M. 1978. The rabbit pulmonary monooxygenase system: Characteristics and activities of two forms of pulmonary cytochrome P-450. Chem. Biol. Interact. 21: 29-43.

POLYCHLORINATED BIPHENYLS: EFFECTS OF STRUCTURE ON BIOLOGIC AND TOXIC ACTIVITY

S. Safe[a,c], A. Parkinson[b], L. Robertson[c], L. Safe[a] and S. Bandiera[a]

ABSTRACT

A number of highly purified synthetic polychlorinated biphenyl (PCB) isomers and congeners were administered to immature male Wistar rats. The effects of this in vivo treatment on the drug-metabolizing enzymes were determined by measuring the microsomal benzo(a)pyrene hydroxylase, 4-dimethylaminoantipyrine N-demethylase and NADPH-cytochrome c reductase enzyme activities, the cytochrome b_5 content and the relative peak intensities and spectral shifts of the reduced microsomal cytochrome P-450: carbon monoxide- and ethylisocyanide-binding difference spectra. The results were compared with the effects of administering phenobarbitone (PB), 3-methylcholanthrene (MC) and PB plus MC (coadministered) to the test animals. The synthetic PCB congeners were selected to test previously reported structure-activity rules for PCBs as hepatic microsomal drug-metabolizing enzyme inducers.

Several PCB isomers and congeners containing only one ortho chlorine resembled PB in their ability to induce cytochrome P-450. These PCBs included 2,3',4,4'-tetra-, 2',3,4,4',5-penta-, 2,3,3',4,4'-penta, 2,3',4,4',5-penta-, 2',3,3',4,4',5-hexa-, 2',3,4,4',5,5'-hexa-, 2,3,3',4,4',5-hexa- and 2,3,3',4,4',5,5'-heptachlorobiphenyl. Similarly, 2,3,3',4',5,6-hexa- and 2,3,3',5,5',6-hexa-, which contain one and no para chlorines, respectively, were PB-type inducers of cytochrome P-450. This indicates that, in contrast to previously reported structure-activity rules, ortho and para chlorination of both phenyl rings is not an absolute requirement for cytochrome P-450 induction. Structure-activity rules for the induction of PB-type activity could not be clearly defined because of the structurally-diverse PCB isomers and congeners which fall into this category.

It was originally proposed that MC-type inducers of cytochrome P-448 require meta and para chlorines on both phenyl rings, a

---

[a] The Guelph-Waterloo Centre for Graduate Work in Chemistry, Department of Chemistry, University of Guelph, Guelph, Ontario.

[b] Department of Biochemistry and Drug Metabolism, Hoffman-LaRoche Inc. Nutley, New Jersey.

[c] Department of Physiology and Pharmacology, College of Veterinary Medicine, Texas A & M University, College Station, Texas.

condition satisfied only by 3,3',4,4'-tetra-, 3,3',4,4',5-penta- and 3,3'4,4',5,5'-hexachlorobiphenyl. However, 3,4,4',5-tetrachlorobiphenyl was identified as a fourth strictly MC-type inducer indicating that the two meta chlorines need not be on different phenyl rings. None of the tested PCBs lacking chlorines in both phenyl rings induced cytochrome P-448.

In contrast to previously proposed structure-activity rules, the addition of one ortho chlorine to the four strictly MC-type PCB inducers failed to abolish their ability to induce cytochrome P-448 (with one exception) and produced a series of seven mixed (PB + MC)-type inducers, 2,3,3',4,4'-, 2,3',4,4',5-, 2',3,4,4',5- and 2,3,4,4',5-pentachlorobiphenyl, 2,3,3'4,4',5'- and 2,3,3',4,4',5-hexachlorobiphenyl and 2,3,3',4,4',5,5'-heptachlorobiphenyl. The one exception, 2',3,4,4',5,5'-hexachlorobiphenyl was shown to closely resemble the novel cytochrome P-450 inducer, isosafrole, in its mode of induction. In addition, all of the mono-ortho substituted PCB congeners reversibly bind to the cytosolic receptor protein which has a high affinity for halogenated aromatics such as 2,3,7,8-tetrachlorodibenzo-p-dioxin which are potent MC-type inducers.

In certain cases (namely 2,3,4,4',5,6-, 2,2',3,3',4,4'-, 2,2',3',4,4',5-, 2,3,3',4,4',6-hexachlorobiphenyl and 2,2',3,3',4,4',5-heptachlorobiphenyl) the addition of two ortho chlorines to the four MC-type PCB congeners failed to abolish their ability to induce cytochrome P-448. It is concluded that PCBs which induce MC-type activity must be substituted in both para positions, at least two meta positions (but not necessarily on different phenyl rings) and can also contain one or two ortho chlorines, particularly if one phenyl ring contains a 2,3,4-trichloro substitution pattern.

Unlike the previously identified MC-type inducers, many of the mixed-type inducers are major or minor components of the commercial PCB preparations and must contribute to their activity as MC-type inducers. Since many of the mixed-type PCB inducers exhibit unusually high toxicity, it is possible that these components are major contributors to both the biologic and toxicologic properties of commercial PCB mixtures.

POLYCHLOROBIPHENYLES: EFFETS DE LA STRUCTURE SUR L'ACTIVITE BIOLOGIQUE ET TOXIQUE

S. Safe[a,c], A. Parkinson[b], L. Robertson[c], L. Safe[a], et S. Bandiera[a]

RESUME

Un certain nombre d'isomères et de congénères hautement purifiés de polychlorobiphényles synthétiques ont été administrés à des rats Wistar mâles immatures. Les effets de ce traitement in vivo sur les enzymes métabolisantes des toxiques ont été déterminés par la mesure de l'activité des enzymes microsomiques benzo(a)pyrène-hydroxylase, 4-diméthylaminoantipyrine-N-déméthylase et NADPH-cytochrome-c-réductase, la teneur en cytochrome b_5 et les intensités des pics et déplacements spectraux relatifs des spectres de différence de la fixation du cytochrome P-450 réduit par le monoxyde de carbone ou l'éthylisocyanure. Les résultats ont été comparés aux effets de l'administration de phénobarbitone (PB), de 3-méthylcholanthrène (MC) et de PB + MC aux animaux d'essai. Les congénères synthétiques des PCB ont été choisis pour tester certaines règles censées lier l'activité et la structure des PCB, en tant que inducteurs d'enzymes métabolisantes des toxiques au niveau des microsomes hépatiques.

Plusieurs isomères et congénères de PCB ne contenant qu'un chlore en ortho ressemblaient à PB en ce qui concerne l'aptitude à induire le cytochrome P-450. Ces PCB étaient les 2,3',4,4'-tétra, 2',3,4,4',5-penta; 2,3,4,4',5-penta; 2,3,3',4,4'-penta; 2,3,4,4',5-penta; 2',3,3',4,4',5-hexa; 2',3,4,4',5,5'-hexa et 2,3,3',4,4',5,5'-hepta. De même, les 2,3,3'4',5,6-hexa et 2,3,3',5,5',6-hexa qui contiennent respectivement un chlore et pas de chlore en para étaient des inducteurs de type PB du cytochrome P-450. Ceci indique que, contrairement aux règles précédemment signalées, la chloration en ortho et para des deux cycles n'est pas absolument nécessaire pour induire le cytochrome P-450. Des règles structure/activité d'induction de l'activité de type PB n'ont pu être définies en raison des variations structurales des isomères et congénères de PCB qui tombent dans cette catégorie.

---

[a] The Guelph-Waterloo Centre for Graduate Work in Chemistry, département de chimie, Université de Guelph, Guelph (Ontario).

[b] Department of Biochemistry and Drug Metabolism, Hoffman-LaRoche Inc. Nutley, New Jersey.

[c] Department of Physiology and Pharmacology, College of Veterinary Medicine, Texas A & M University, College Station, Texas.

A l'origine on avait prétendu que les inducteurs de type MC du cytochrome P-448 devaient être chlorés en méta et para sur les deux cycles, une condition que ne remplissent que 3,3',4,4'-tétra; 3,3',4,4',5-penta; et 3,3',4,4',5,5'-hexa. Toutefois, le 3,4,4',5-tétrachlorobiphényle s'est révélé un quatrième inducteur de type strictement MC, ce qui montre que les deux chlore en méta n'ont pas nécessairement à être sur deux cycles. Aucun des PCB sans chlore en para sur les deux cycles n'induisait le cytochrome P-448.

Contrairement aux règles structure/activité précédemment invoquées, l'addition d'un chlore en ortho sur les quatre PCB inducteurs de type strictement MC n'a pas aboli leur pouvoir d'induction du cytochrome P-448 (à une exception près) et a produit une série de sept inducteurs mixtes de type (PB + MC), à savoir: 2,3,3',4,4'-penta; 2,3',4,4',5-penta; 2',3,4,4',5-penta; 2,3,4,4',5-penta; 2,3,3',4,4',5'-hexa; 2,3,3',4,4',5-hexa et 2,3,3',4,4',5,5'-hepta. On a montré que l'exception, le 2',3,4,4',5,5'-hexachlorobiphényle, ressemblait étroitement au nouvel inducteur du cytochrome P-450, l'isosafrole, dans son mode d'induction. De plus, tous les congénères des PCB mono-ortho se liaient de façon réversible à la protéine réceptrice cytosolique qui a une forte affinité pour les aromatiques halogénés, tels que la 2,3,7,8-tétrachlorodibenzo-p-dioxine, qui sont de puissants inducteurs de type MC.

Dans certains cas (à savoir les PCB 2,3,4,4',5,6-hexa; 2,2',3,3',4,4'-hexa; 2,2',3,3',4,4',5-hexa; 2,3,3',4,4',6-hexa et 2,2',3,3',4,4',5-hepta) l'addition de deux chlore en ortho sur les quatre PCB inducteurs de type MC n'abolissait pas leur capacité d'induction du cytochrome P-448. On en a conclu que les PCB induisant une activité de type MC devaient être substitués aux deux positions para, à au moins deux des positions méta (mais pas nécessairement sur deux cycles différents) et pouvaient porter aussi un ou deux chlore en ortho, particulièrement si un des cycles avait la configuration 2,3,4-trichloro.

A la différence des inducteurs de type MC précédemment identifiés, beaucoup des inducteurs de type mixte sont des constituants importants ou secondaires des préparations commerciales de PCB et ils doivent contribuer à leur activité d'inducteurs de type MC. Etant donné que beaucoup des PCB inducteurs de type mixte sont très toxiques, il est possible que ces composants contribuent de façon importante aux propriétés biologiques et toxicologiques des mélanges commerciaux de PCB.

INTRODUCTION

Commercial polychlorinated biphenyls (PCBs) have been widely used in industry as heat transfer fluids, hydraulic fluids, solvent extenders, plasticizers and as dielectric fluids in electrical capacitors and transformers (Hutzinger et al. 1974). The commercial PCBs are prepared by the direct chlorination of biphenyl and the properties and applications of the commercial products are dependent on their degree of chlorination. PCBs have been marketed world-wide under diverse trade names (Aroclor, Clophen, Phenoclor, Kanechlor, Santotherm and Fenclor).

The diverse industrial applications of PCBs are directly related to their physical and chemical properties which include (1) resistence to acids and bases; (2) resistance to oxidation and reduction; (3) solubility in organic solvents; (4) excellent dielectric properties (as electrical insulators); (5) thermal stability and (6) non-flammability. Unfortunately, many of these properties are major factors in the persistence of PCBs in the environment.

Commercial PCBs are not simple products but complex mixtures of various isomers and congeners. Although there are 209 possible individual PCB congeners, most of the commercial products are mixtures of 50 or more compounds as indicated by capillary gas chromatographic analysis (Sissons andd Welti 1971; Jensen and Sundström 1974; Ballschmitter and Zell 1980).

The first report of PCBs in human samples (Jensen and Sundström 1974) stated that "PCB...was detected...in his own, his wife's and his baby daughter's hair. As the baby is only five months old her father concludes that she got her dose of PCB with her mother's milk." Numerous studies throughout the world indicate that the detection frequency of PCBs in human tissue is high and the residue levels are exceeded in concentration only by DDE (Mes et al. 1977; Holdrinet et al. 1977; Landrigan 1980). A summary of representive PCB residue levels in human tissue is given in Table 1.

A major development which will permit the assessment of the health impact of PCBs in humans has been the identification of the major congeneric constituents by a combination of GC, GC-MS and comparative GC analysis using synthetic standards. Table 2 summarizes the major PCB congeners identified in Japanese human tissue and their average concentrations in breast milk from women in the Osaka Prefecture (Watanabe et al. 1979). Recently we have synthesized the major breast milk PCB congeners and have prepared a reconstituted breast milk PCB mixture (Table 2). Moreover the capillary gas chromatogram of this synthetic mixture was quantitatively and qualitatively similar to that of a composite North American breast milk PCB sample (courtesy Dr. J. Mes, Health and Welfare Canada, Ottawa, Ontario and Dr. M. Mullin, Environmental Protection Agency, Grosse Ile, Michigan, unpublished

Table 1. PCBs in human tissue.

Country	PCB Concentration ($mg.g^{-1}$ in fat*) Adipose* Tissue	Breast Milk	Blood ($\mu g.g^{-1}$)
United States	0.4-3.5	detected	-
Canada	0.6-11.0	1.2	-
	0.10-0.61	0.48	
Japan		1.1±0.4	2.5-4.2
Scandinavia	0.88-2.14	0.8-0.9	3.1-12.0

Table 2. Relative concentration of PCB congeners in Japanese breast milk and in a reconstituted PCB mixture.

PCB Structure	PCB Concentration in Japanese Breast Milk (%)	PCB Concentration in Reconstituted Breast Milk Mixture (%)
2,4,4'-TCBP	8.4 ± 2.2	9.2
2,2',5,5'-TCBP	2.0 ± 1.3	2.2
2,4,4',5-TCBP	19.1 ± 2.6	20.1
2,2',4,5,5'-PCBP	2.8 ± 0.9	3.0
2,3',4,4',5-PCBP	11.8 ± 1.2	12.8
2,2',3,4',5,5'-HCBP	2.3 ± 0.3	-
2,3,3',4,4'-PCBP	3.5 ± 0.5	3.9
2,2',4,4',5,5'-HCBP	15.5 ± 0.4	16.3
2,2',3,4,4',5'-HCBP	15.8 ± 0.7	16.9
2,2',3,4',5,5',6-HCBP	3.2 ± 0.6	3.3
2,2',3,4,4',5',6-HCBP	1.6 ± 0.3	1.7
2,3,3',4,4',5-HCBP	2.1 ± 0.7	2.3
2,2',3,4,4',5,5'-HCBP	5.1 ± 0.7	5.7
2,2',3,3',4,4',5-HCBP	2.3 ± 0.3	2.3
Total	95.5	99.9

results). This reflects the global nature of the commercial product and its bioconcentration in humans from diverse locations.

EFFECTS OF STRUCTURE ON THE BIOLOGIC ACTIVITY OF PCBs AS MICROSOMAL ENZYME INDUCERS

One of the most sensitive and easily measured biochemical responses to halogenated hydrocarbons is the induction of hepatic microsomal, cytochrome P-450-dependent, monooxygenases. Inducers of these drug-metabolizing enzymes have been divided into three main categories, namely phenobarbitone (PB)-type inducers of cytochrome P-450, 3-methylcholanthrene (MC)-type inducers of cytochrome P-448 and mixed (PB + MC)-type inducers. Polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-p-dioxins (PCDDs), notably 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), are MC-type inducers of cytochrome P-448-dependent monoxygenases, e.g., aryl hydrocarbon hydroxylase (AHH), whereas commercial PCB mixtures, e.g. Aroclor 1254, and PBB mixtures, e.g. fireMaster FF-1, are potent mixed-typed inducers of both cytochromes.

It has been proposed that the PB-type and MC-type characteristics of Aroclor 1254 are dependent on the presence of individual PCB congeners, some of which induce cytochrome P-450 and some of which induce cytochrome P-448. In support of this proposal, several individual chlorinated biphenyls were identified as inducers of rat hepatic microsomal drug-metabolizing enzymes (Fujita et al. 1971; Chen et al. 1973; Johnstone et al. 1974; Ecobichon and Comeau 1975; Ecobichon et al. 1977) and were subsequently categorized into PB-type inducers (Stonard and Greig 1976; Alvares 1977; Goldstein et al. 1977; Poland and Glover 1977; Yoshimura et al. 1978; Goldstein 1979; Kohli et al. 1980). From these studies on the inductive effects of individual PCB congeners the following structure-activity rules were proposed:

Rule 1. Biphenyl chlorinated in the ortho and para positions of both phenyl rings is a PB-type inducer of cytochrome P-450-dependent monooxygenases as exemplified by 2,2',4,4'-tetrachlorobiphenyl. Inducibility is not altered by further ortho or meta chlorination as indicated by the PB-type inducers 2,2',4,4',6,6'-hexachlorobiphenyl and 2,2',4,4',5,5'-hexachlorobiphenyl, respectively.

Rule 2. Biphenyl chlorinated in only the meta and para positions of both phenyl rings is an MC-type inducer of cytochrome P-488-dependent monooxygenases, as exemplified by 3,3'4,4'-tetrachlorobiphenyl. Inducibility is abolished by ortho chlorination, as indicated by the PB-type inducer, 2,2',4,4',5,5'-hexachlorobiphenyl. In contrast, further meta chlorination of 3,3',4,4'-tetrachlorobiphenyl does not alter its ability to induce cytochrome P-448, as indicated by the MC-type inducers, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl.

Rule 3. Most of the lower chlorinated biphenyls including biphenyl itself possess little or no inducing activity.

In certain reactions, such as the Ullman coupling of chloroiodobenzenes, the synthesis of chlorinated biphenyls can give rise to PCDFs (Moron et al. 1973), some of which are over 100 times more active than the most potent PCB congeners as inducers of cytochrome P-448 (Poland and Glover 1977; Poland et al. 1979). A dramatic consequence of contamination with PCDFs was illustrated for a commercially-available, 99% pure 2,2',4,4',5,5'-hexachlorobiphenyl (Goldstein et al. 1978). The reported ability of this congener to induce cytochrome P-448 (Alvares 1977) was credited entirely to trace quantities (1%) of four PCDF congeners, including 44 mg/g of the most potent cytochrome P-448 inducer, 2,3,7,8-tetrachlorodibenzofuran (TCDF). Two other di-ortho substituted PCB congeners, namely 2,2',3,3',4,4'-hexa- and 2,2',3',4,4',5-hexachlorobiphenyl, have been reported to induce cytochrome P-448 (Stonard and Greig 1976). However, like 2,2',4,4',5,5'-hexachlorobiphenyl, these congeners were synthesized by the Ullman reaction and, hence, were possibly contaminated with TCDF-like impurities. Because of this possibility, the reported ability of the di-ortho substituted PCBs to induce cytochrome P-448 is not regarded as a contradiction to Rule 2 (Goldstein 1979).

The structure-activity rules proposed for PCBs as inducers of multiple forms of cytochrome P-450 are compatible with the following four observations. First, a comparison of Aroclors 1221, 1232, 1242, 1248, 1254 and 1260, which contain 21%, 32%, 42%, 48%, 54% and 60% chlorine by weight, respectively, revealed that the potency of these PCB mixtures as drug-metabolizing enzyme inducers increased with increasing chlorine content (Ecobichon and Comeau 1974). The most potent inducers, namely Aroclors 1248, 1254 and 1260, contain predominantly tetra-, penta- and hexachlorobiphenyl isomers, respectively. Further evidence that the tetra-, penta- and hexachlorobiphenyl isomers are major contributors to the inducing properties of commercial PCB mixtures was provided by Goldstein et al. (1975) who compared the biologic and toxicological effects produced by Aroclor 1016 and Aroclor 1242 (which contain 41% and 42% chlorine, respectively). Aroclor 1242, which is composed of 25% tetra-, 8% penta- and 1% hexachlorobiphenyls, was a potent inducer of rat hepatic microsomal drug-metabolizing enzymes and caused hepatic porphyria. In contrast, Aroclor 1016, which is composed of 21% tetra-, 1% penta- and 0.1% hexachlorobiphenyls exerted minimal effects on the drug-metabolizing enzymes and was porphyrinogenic only at extremely high doses. Since the difference between Aroclor 1016 and 1242 could not be attributed to either pharmacodynamic differences or differences in the levels of contaminating PCDFs which are nonporphyrinogenic (Goldstein et al. 1976), it was concluded that the higher percentage of tetra-, penta- and hexachlorobiphenyls in Aroclor 1242 are responsible for the greater biologic and toxicologic potency of this PCB mixture (Goldstein et al. 1975).

Second, the three MC-type PCB congeners defined in Rule 2, namely 3,3',4,4'-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl, are approximately isosteric with the most potent MC-type inducers, 2,3,7,8-TCDD and TCDF (Poland and Glover 1977), i.e.,

they are chlorinated in the lateral positions of the biphenyl nucleus and without the steric hindrance of ortho chlorines can readily assume a coplanar configuration. These stereochemical features apparently favor the binding of halogenated aryl hydrocarbons to a high-affinity, low-capacity cytosolic protein believed to be the receptor for the induction of cytochrome P-448 (Poland et al. 1979). The apparent requirement for coplanarity of the two phenyl rings is suggested not only by the lack of ortho chlorines from the three MC-type PCB congeners, but also by the fact that conversion of 3,3',4,4'-tetrachlorobiphenyl to the rigid, planar analogue, 2,3,6,7-tetrachlorobiphenylene, increases its potency both as a cytochrome P-448 inducer and as a receptor binder by about 100-fold (Poland and Glover 1977). In contrast to 2,3,7,8-TCDD, but like the polycyclic aryl hydrocarbons, 3,3',4,4'-tetrachlorobiphenyl (Poland and Glover 1977) and 3,3',4,4'5,5'-hexachlorobiphenyl (Kohli et al. 1980) are not sufficiently potent to induce cytochrome P-448 in the DBA/2 genetically nonresponsive strain of mouse.

Third, the cytochrome P-450 inducer, 2,2',4,4',5,5'-hexachlorobiphenyl, further resembles PB in its ability to induce NADPH-cytochrome c reductase, glutathione S-transferase and epoxide hydrolase while the cytochrome P-448 inducer, 3,3',4,4',5,5'-hexachlorobiphenyl, further resembles MC in its ability to induce glutathione S-transferase and UDP-glucuronosyl transferase (Goldstein et al. 1977; Kohli et al. 1979b, 1980). Fourth, the structure-activity rules, together with limited toxicological data (Goldstein et al. 1976; McKinney et al. 1976; Kawanishi et al. 1978; Kohli et al. 1979a) suggest that, like several other classes of halogenated aryl hydrocarbons, the toxicity of the individual PCB congeners correlates with their potency as AHH inducers (Poland et al. 1979).

However, the proposed structure-activity rules are incompatible with a very important observation, namely that the three possible MC-type PCB congeners defined in Rule 2 are undetectable in Aroclor 1254, as might be expected since meta chlorination of biphenyl is less favorable than both ortho and para chlorination (Ballschmitter and Zell 1980). The absence of these three congeners raises the question: What components in Aroclor 1254 induce cytochrome P-448? In view of the apparent correlation between toxicity and cytochrome P-448 induction, the identification of the MC-type inducers in Aroclor 1254 may simultaneously identify the more toxic components of this environmental contaminant. For this reason, the structure-activity relationship for PCBs as inducers of multiple forms of cytochrome P-450 was reinvestigated (Parkinson et al. 1980a-e, 1981).

REVISIONS

STRUCTURE-ACTIVITY RULES: PB-TYPE INDUCERS

The structural requirement for both para substituents was tested by evaluating the effects of a series of three hexachlorobiphenyl

isomers, 2,3,3',4,4',5-hexachlorobiphenyl (HCBP-1), 2,3,3',4',5,6-hexachlorobiphenyl (HCBP-2) and 2,3,3'5,5',6-hexachlorobiphenyl (HCBP-3), as microsomal enzyme inducers. We have previously reported that HCBP-1 (with two para substituents) was a mixed-type inducer and it is apparent from the results that pretreatment of rats with either HCBP-2 (with one para substituent) or HCBP-3 (with no para substituents) resulted in a PB-type enhancement of hepatic microsomal benzo(a)pyrene hydroxylase and dimethyl aminoantipyrene N-demethylase activities and cytochrome P-450 content. A fourth isomer devoid of para substituents, 2,2',3,5,5',6-hexachlorobiphenyl, exhibited only marginal PB-type activity. These data and previous results reported for 2,2',3,3',5,5'-hexachlorobiphenyl (Kohli et al. 1979a,b) demonstrates that para chloro substitution of biphenyl is not a structural requirement for PB-type induction.

It has also been reported that administration of 4,4'-dichlorobiphenyl to rats resulted in proliferation of hepatic smooth endoplasmic reticulum and increases in hepatic microsomal aniline hydroxylase and p-nitroanisole-O-demethylase activities (Ecobichon et al. 1977). These data suggested that PCB congeners without any ortho chloro substituents can be PB-type inducers. The activity observed for 4,4'-dichlorobiphenyl may be associated with one or more of its metabolites. However, in the rat, the major metabolites of 4,4'-dichlorobiphenyl are 4,4'-dichloro-3-biphenylol and 4'-chloro-4-biphenylol (Sundström et al. 1976), both of which lack ortho substituents. Furthermore, 3,3'-dichlorobiphenyl is also a weak PB-type inducer (Goldstein et al. 1977). Because of such variability it is apparent that the precise structural requirements for PCBs as PB-type inducers of microsomal enzyme activities cannot be readily defined.

STRUCTURE ACTIVITY RULES: MC-TYPE INDUCERS

It was originally proposed that MC-type inducers require only para substitution and meta substitution on both phenyl rings. However, the requirements for meta substitution have not been unequivocally established. It has been shown that all mixed or MC-type inducers were substituted at one or two meta positions on both phenyl rings. We have also shown that 3,4,4',5-tetrachlorobiphenyl is also a pure MC-type inducer, thus indicating that PCB congeners with pure MC-type activity must be substituted with at least two meta chloro substituents, but not necessarily on the same ring. A reinvestigation of the requirements for para substitution confirmed that para chloro substitution on both phenyl rings was a structural prerequisite for PCBs with MC-type activity. For example, removal of the C-4' chlorine of the mixed-type inducer, 2,3,3',4,4',5,5'-heptachlorobiphenyl or transfer of the C-4' chlorine of the mixed-type inducer, 2,3,3',4,4',5'-hexachlorobiphenyl, to the C-5' position gives 2,3,3',4,5,5'-hexachlorobiphenyl which induces cytochrome P-450 and not cytochrome P-448.

We have also systematically investigated the effects of chloro substituents on the activity of all four MC-type inducers by preparing and testing all their possible mono-ortho analogs (Figure 1). With one exception, namely 2',3,4,4',5,5'-hexachlorobiphenyl, all of these compounds were mixed-type inducers. Presumably, the steric properties of only one ortho chloro substituent are not sufficient to hinder the formation of a coplanar PCB moiety which is isosteric with 2,3,7,8-TCDD and binds to the cytosol receptor protein involved in the initiation of microsomal monooxygenase enzyme induction. The lack of mixed-typed activity for 2,3',4,4',5,5'-hexachlorobiphenyl was surprising since the bromo analog was reported to be a mixed inducer (Dannan et al. 1978).

Two ortho chloro substituents might be expected to have a more pronounced effect on the population of the planar conformers and, hence, on their activity as MC-type inducers. In an attempt to resolve conflicting reports in the literature, the effects of 2,2',3,3',4,4'-hexa-, 2,2',4,4',5,5'-hexa- and 2,2',3',4,4',5-hexachlorobiphenyl on the hepatic microsomal drug-metabolizing enzymes were evaluated in the immature male rat. By comparison with the effects of the classical enzyme inducers, PB and MC, 2,2',4,4',5,5'-hexachlorobiphenyl was classified as a pure PB-type inducer. In contrast, 2,2',3,3',4,4'-hexachlorobiphenyl, irrespective of its synthetic route, exhibited PB-type and weak MC-type characteristics; the most prominent feature of the latter was a 7-fold increase in 4-chlorobiphenyl hydroxylase activity. 2,2',3',4,4',5-Hexachlorobiphenyl also resembled a mixed (PB + MC)-type inducer although its MC-type characteristics were more pronounced that those of 2,2'3,3',4,4'-hexachlorobiphenyl. Since these three hexachlorobiphenyls are disubstituted derivatives of the pure MC-type inducer, 3,3',4,4'-tetrachlorobiphenyl, it is clear that the presence of two ortho chlorines does not necessarily abolish MC-type character. The addition of two ortho chlorines to the four MC-type PCB inducers (e.g. Figure 2) gave four mixed-type inducers all containing a 2,3,4-trichloro substitution pattern (2,2',3,3',4,4'-, 2,2',3,4,4',5'-, 2,3,3',4,4',6- and 2,2',3,3',4,4',5-hexachlorobiphenyl). In addition, the effects of 2,3,4,4',5,6-hexachlorobiphenyl were also consistent with a mixed-type induction pattern.

We conclude that PCBs which induce MC-type activity must be substituted in both para positions, at least two meta positions (but not necessarily on different phenyl rings) and can also contain one or two ortho chlorines, particularly if one phenyl ring contains a 2,3,4-trichloro substitution pattern.

ACTIVITY OF BREAST MILK PCBs

Table 3 summarizes all the PCBs expected to exhibit MC-type activity based on the above guidelines. A number of these isomers and congeners have been identified in commercial PCBs and in humans (blood, adipose tissue and breast milk). As noted in Table 2, there is a relatively high concentration of MC-type inducers which preferentially

PARENT
MC-TYPE INDUCERS

MONO-*ORTHO* DERIVATIVES

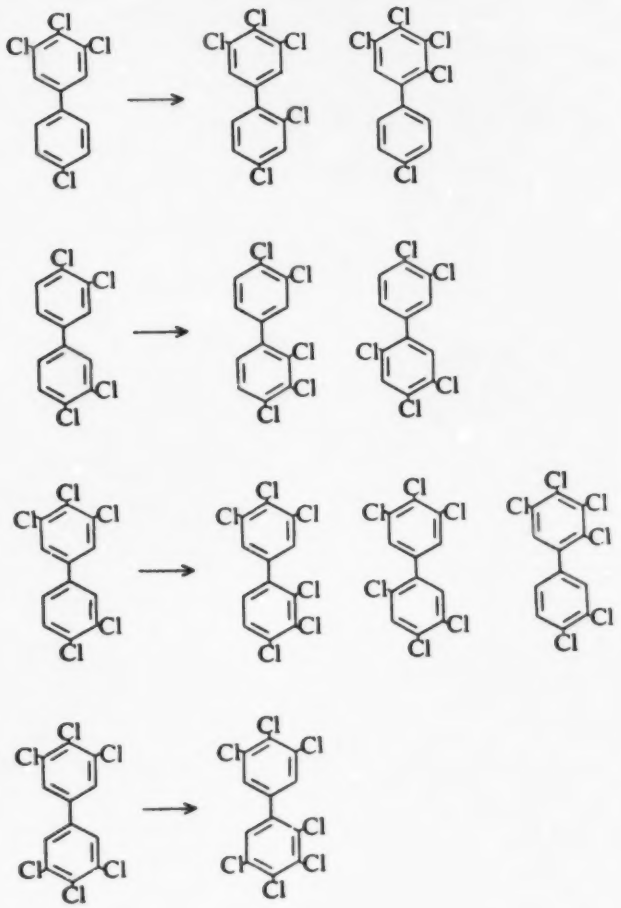


Figure 1. Structures of the MC-type PCB inducers and all their mono-ortho chloro substituted congeners.

PARENT
MC-TYPE INDUCERS

DI-*ORTHO* DERIVATIVES

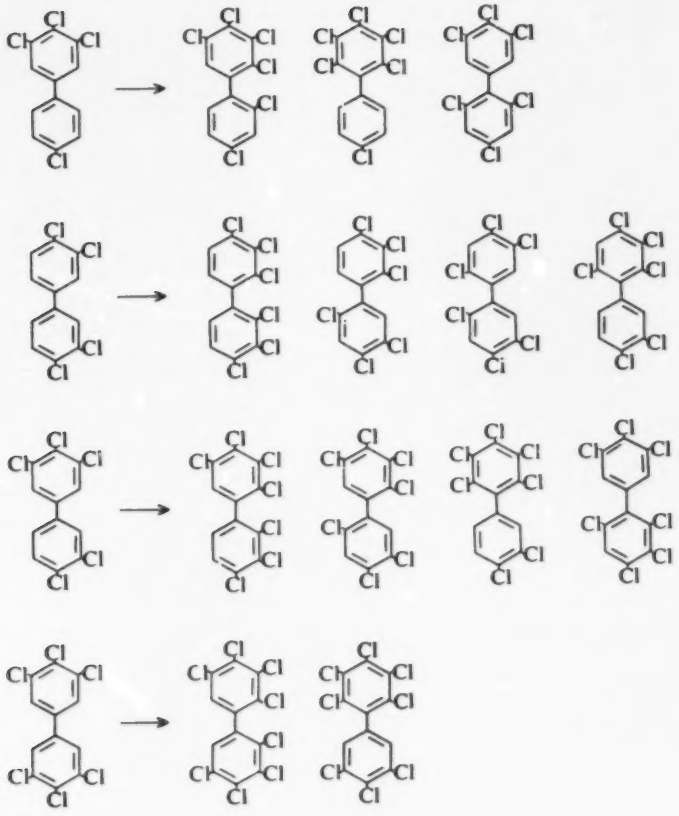


Figure 2. Structures of the MC-type PCB inducers and all their di-ortho chloro substituted congeners.

Table 3. Identification in Aroclor 1254 of the PCB congeners tested as microsomal enzyme inducers.

Chlorinated Biphenyl	Type of Inducer	Presence in Aroclor 1254
Parent MC-type inducers		
3,4,4',5-TCBP	MC	ND
3,3',4,4'-TCBP	MC	ND
3,3',4,4',5-PCBP	MC	ND
3,3',4,4',5,5'-HCBP	MC	ND
Mono-ortho derivatives		
2',3,3',4,4'-PCBP	MIXED	ND
2,3,4,4',5-PCBP	MIXED	ND
2,3,3',4,4'-PCBP	MIXED	A
2,3',4,4',5-PCBP	MIXED	A
2',3,3',4,4',5-HCBP	MIXED	ND
2',3,4,4',5,5'-HCBP	PB	B
2,3,3',4,4',5-HCBP	MIXED	C
2,3,3',4,4',5,5'-HpCBP	MIXED	B
Di-ortho derivatives		
2,2',3,4,4',5-HCBP-4	PB	B
2,3,4,4',5,6-HCBP-5	MIXED	ND
2,3',4,4',5,6-HCBP-6	INACTIVE	ND
2,2',3,3',4,4'-HCBP-7	MIXED	C
2,2',3',4,4',5-HCBP-8	MIXED	A
2,2',4,4',5,5'-HCBP-9	PB	A
2,3,3',4,4',6-HCBP-10	MIXED	ND
2,2',3,3',4,4',5-HpCBP-1	MIXED	C
2,2',3,4,4',5,5'-HpCBP-2	PB	C
2,3,3',4,4',5,6-HpCBP-3	PB	ND
2,3,3',4,4',5',6-HpCBP-4	PB	ND
2,2',3,3',4,4',5,5'-OCBP-1	PB	ND
2,3,3',4,4',5,5',6-OCBP-2	PB	ND

ND - Not detected.

A - Major component.

B - Trace component.

C - Minor component.

bioconcentrate in human breast milk. A recent study in our laboratory (Parkinson et al. 1980f) has shown that the dose effecting half-maximal (ED_{50}) induction of AHH activity for a reconstituted breast milk PCB sample was 12 $\mu mol.kg^{-1}$ whereas the ED_{50} for Kanechlor 500, a commercial PCB mixture, was 87 $\mu mol.kg^{-1}$ (Figure 3). Thus, the increased biological potency of breast milk PCBs reflects the preferential bioconcentration of 2,3',4,4',5-penta-, 2,3,3',4,4'-penta-, 2,3,3',4,4',5-hexa- and 2,2',3,3',4,4',5-heptachlorobiphenyl which are mixed-type inducers.

CYTOSOLIC RECEPTOR BINDING

Incubation of rat hepatic cytosol with 10 nM [^{3}H]-2,3,7,8-TCDD for 1 h at 5°C produced a specific binding peak detectable by sucrose density gradient analysis (Okey et al. 1979). The concentration of the receptor in hepatic cytosol was found to be approximately 30 fmol/mg of cytosolic protein. This value was remarkably constant over more than 30 experiments and with different preparations of cytosol from animals of the same age. In the presence of 10 µM nonradiolabelled 2,3,7,8-TCDD or 10 µM 3-methycholanthrene, this peak was completely abolished. In the presence of 10 µM phenobarbitone, however, this peak was unaltered.

Fourteen highly purified, synthetic, chlorinated biphenyl congeners, as well as the commercial mixture, Aroclor 1254, were tested in competition experiments. The results of the competitive binding between 10 nM [^{3}H]-2,3,7,8-TCDD and differing concentrations of the biphenyl congeners were plotted and the EC_{50} for these compounds were determined (Figure 4). Of the compounds which are considered "pure MC-like" inducers, MC was the best competitor with [^{3}H]-2,3,7,8-TCDD for the receptor and completely abolished the binding peak at a concentration of 1 µM. Both MC and 2,3,7,8-TCDD bound more avidly than 3,3',4,4',5-pentachlorobiphenyl, which was found to be the most potent competitor among the chlorinated biphenyl congeners tested and completely inhibited [^{3}H]-2,3,7,8-TCDD binding at a concentration of 10 µM. The next most effective competitor in the PCB series was 3,3',4,4'-tetrachlorobiphenyl. The other two "MC-like" biphenyls, 3,3',4,4',5,5'-hexachlorobiphenyl and 3,4,4',5-tetrachlorobiphenyl, also competed with [^{3}H]-2,3,7,8-TCDD for specific binding sites, but at higher concentrations than 3,3',4,4'-tetrachlorobiphenyl.

All eight of the PCB congeners, as well as the commercial mixture, Aroclor 1254, were very similar in their ability to inhibit binding to the Ah receptor. At concentrations ranging between 0.1 mM and 1.0 mM, these chlorinated biphenyls eliminated the cytosolic binding peak, although for many of the congeners, higher concentrations could not be achieved because of the limited solubilities of the biphenyls both in aqueous solution and in dimethyl sulfoxide.

Of the compounds which are classed as "PB-type" inducers, the two polychlorinated biphenyls, 2,2',4,4'-tetra- and 2,2',4,4',5,5'-

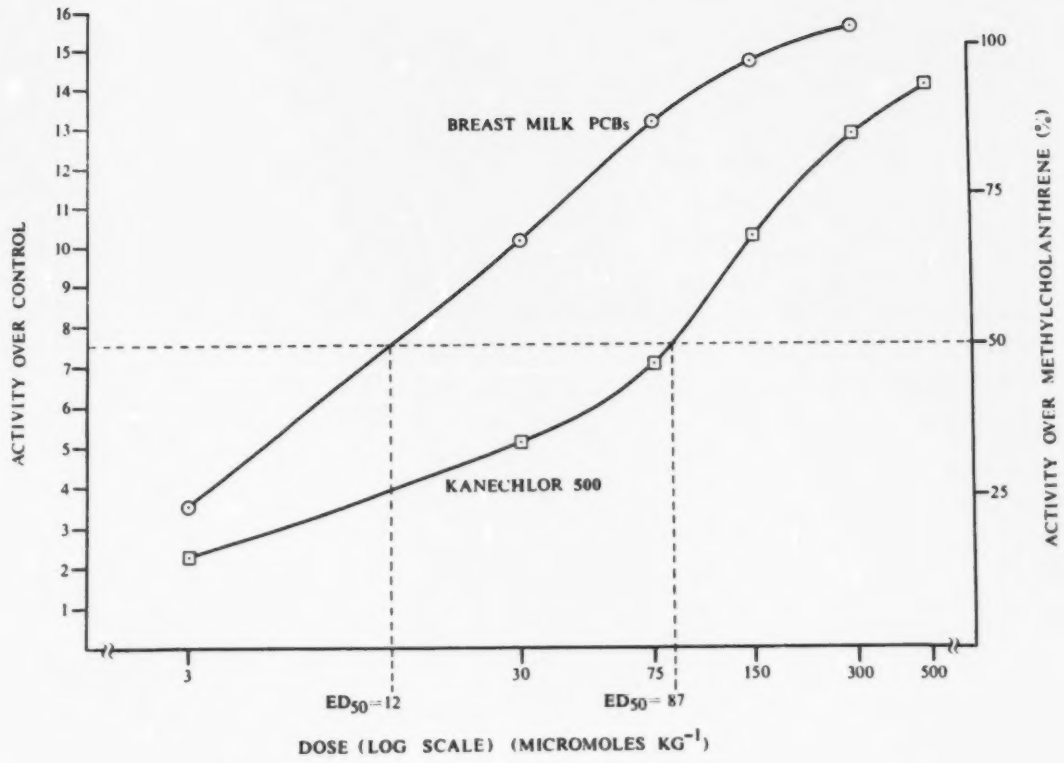


Figure 3. The effects of dose on the activity of Kanechlor 500 and related constituted breast milk PCBs as AHH inducers.

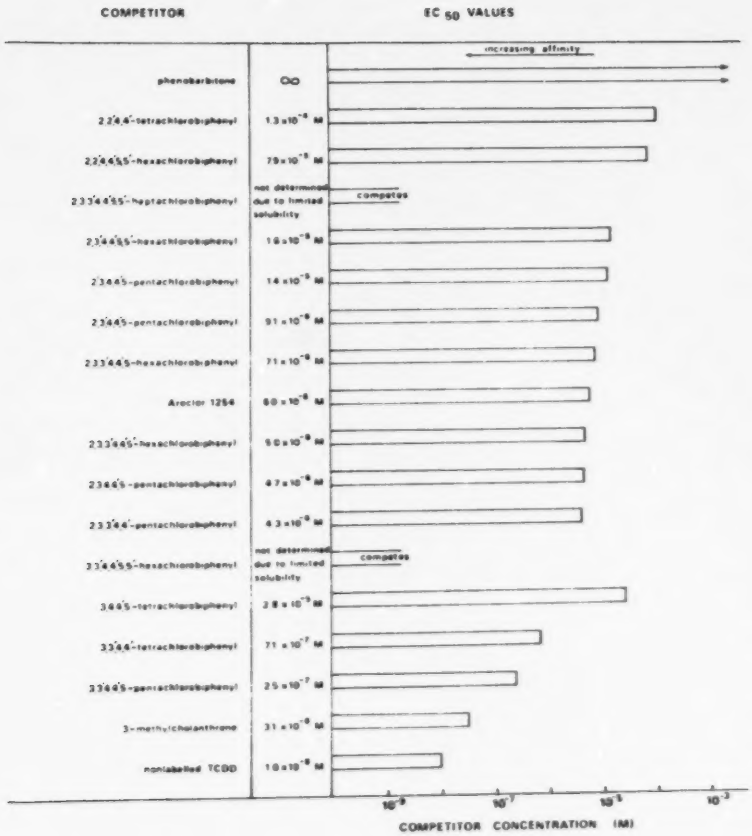


Figure 4. PCB isomers and congeners as competitors with [^{3}H]-2,3,7,8-TCDD for the cytosolic receptor protein.

hexachlorobiphenyl, bound to the receptor as evidenced by the competition with [^{3}H]-2,3,7,8-TCDD at high concentrations (1.0 mM). This finding was somewhat unexpected in view of the fact that these two PCBs did not abolish the [^{3}H]-2,3,7,8-TCDD binding complex at a concentration of 10 μM and, more importantly, are not considered to be AHH inducers, nor to have any "MC-type" character. The fact that they do act as competitors suggests that perhaps their molecular structure approximates that of the MC-type and mixed-type PCBs enough to allow binding to the receptor at high concentrations. PB did not compete with [^{3}H]-2,3,7,8-TCDD for the receptor, even at the highest concentration tested (10 mM).

Thus our experiments with PCB isomers and congeners confirm the correlation previously noted within the PCDD series, between their avidity in binding to the cytosolic receptor protein and their relative potencies as AHH inducers. There is evidence to suggest that like the MC-type PCB inducers, some of the mixed-type inducers are more toxic than the PB-type inducers. For example, administration of the mixed-type inducer, 2,3,3',4,4'-pentachlorobiphenyl to rats as a single oral dose of 185 μmol.kg^{-1} (60 mg.kg^{-1}), caused progressive weight loss, fatty liver and 100% mortality within 8 days, whereas none of these acute toxic effects were observed in rats similarly treated with 500 μmol.kg^{-1} (150 mg.kg^{-1}) of the PB-type inducer, 2,2',4,4',5,5'-hexachlorobiphenyl (Yamamoto et al. 1976).

Ax and Hansen (1975) observed 100% embryo mortality in eggs from chickens fed diets containing 20 mg.kg^{-1} of the mixed-type inducer, 2,3',4,4',5-pentachlorobiphenyl, but only 10% mortality (i.e. control values) for the PB-type inducer, 2,2',4,4',5,5'-hexachlorobiphenyl. From long-term feeding experiments, Stonard and Greig (1976) determined that, in contrast to the PB-type inducer 2,2',4,4',5,5'-hexachlorobiphenyl, the mixed-type inducers, 2,2',3,3',4,4'- and 2,2',3,4,4',5'-hexachlorobiphenyl were porphyrinogenic to female rats. The mixed-type inducers, 2,3,3',4,4'- and 2,3',4,4',5-penta- and 2,3,3',4,4',5-hexachlorobiphenyl, all cause fatty liver and thymic atrophy in rats (Yoshihara et al. 1979) and it was suggested that the toxicity of these and other PCBs correlates with their ability to induce cytochrome P-448 (Yoshimura et al. 1979). It is possible, therefore, that the mixed-type inducers identified in the present study are major contributors to both the biologic and toxicologic properties of the commercial PCB mixtures and breast milk PCBs.

PCBs AND RELATED COMPOUNDS - UNANSWERED PROBLEMS

The effects of structure on the biologic and toxic activity of PCB isomers and congeners complement similar data obtained for the PCDDs, and support the hypothesis for the mechanism of action of the halogenated aromatics (Poland and Glover 1980; Poland et al. 1979). In order to further test the validity of the proposed mechanism, several additional studies are in progress in our laboratory and these include:

(1) determining the comparative toxicities of MC-, PB and mixed-type PCB inducers;
(2) evaluating the effects of mixed-type PCBs on responsive (C57BL/6J) and nonresponsive (DBA/2J) mice;
(3) determining the effects of structure on the biologic and toxic activities of polybrominated biphenyls and naphthalenes, polychlorinated naphthalenes, terphenyls and azobenzenes;
(4) evaluating the biologic and toxic activities of individual PCB congeners and reconstituted mixtures (e.g. "breast milk PCBs") to assess possible additive, synergistic or antagonistic effects of mixtures;
(5) assessing the role of PCBs as promoters or inhibitors of chemical carcinogenesis.

Another major question which remains unanswered concerns the precise structural requirements for binding to the cytosolic receptor protein. Both 2,2',4,4',5,5'-hexachlorobiphenyl, a PB-type inducer and 2,3',4,4',5,5'-hexachlorobiphenyl, a novel-type inducer, bind to this receptor whereas PB does not bind at all. Is this due to nonspecific binding or are these PCB isomers weak inducers of cytochrome P-448-dependent activity? These questions, as yet, remain unanswered. McKinney and coworkers have recently proposed a unifying hypothesis which attempts to explain the structure-activity relationships in halogenated biphenyls (McKinney and Singh 1981). This proposal requires not only maximum coplanarity of the biphenyl rings but also a net polarizability in both lateral positions (i.e., 3,4 and 3',4'). This theory presupposes that interaction between the hydrophobic biphenyl ligand and the cytosolic receptor protein depends, in part, on a favorable dipole-dipole interaction. Minimal structural conditions for this interaction require at least two chlorine atoms at the lateral position in each phenyl ring; one bromine and two bromine (or chlorine) atoms in each phenyl ring; and presumably replacement of the halogen atoms with an iodo group would not diminish this type of induction activity. Recently we have shown that 3,4-dichloro-4'-iodo- and 4'-bromobiphenyl induce hepatic cytochrome P-448-dependent monooxygenase enzyme activity, supporting McKinney and Singh's hypothesis. However, 3,4,4'-trichlorobiphenyl was inactive and 3,4-dichloro-4'-fluorobiphenyl was active as an AHH inducer and these results do not support the proposed hypothesis. Moreover, the potent activity of 2,3,4,4',5-pentachlorobiphenyl (with only one Cl group on the lateral position of one of the phenyl rings) as an AHH inducer contradicts the unifying hypothesis. Research in progress in our laboratory is attempting to more fully evaluate the structural prerequisites (for halogenated aromatics) necessary for binding to the cytosolic receptor protein and microsomal AHH activity.

ACKNOWLEDGEMENTS

The financial assistance of Health and Welfare Canada, Research Programs Directorate, the Natural Sciences and Engineering Research Council and the National Institute of Health (1-R01-ES02798-01) is gratefully appreciated.

REFERENCES

Alvares, A.P. 1977. Stimulatory effects of polychlorinated biphenyls (PCB) on cytochrome P-450 and P-448 mediated microsomal oxidations. In Microsomes and drug oxidations. Edited by V. Ullrich. Pergamon Press, New York. pp. 476-483.

Ax, R.L. and Hansen, L.G. 1975. Effects of purified PCB analogs on chicken reproduction. Poult. Sci. 54: 895-900.

Ballschmitter, K. and Zell, M. 1980. Analysis of polychlorinated biphenyls (PCB) by glass capillary gas chromatography. Composition of technical Aroclor and Clophen-PCB mixtures. Fresenius Z. Anal. Chem. 302: 20-31.

Chen, P.R., Mehendale, H.M. and Fishbein, L. 1973. Effect of two isomeric tetrachlorobiphenyls on rats and their hepatic enzymes. Arch. Environ. Contam. Toxicol. 1: 36-47.

Dannan, G.A., Moore, R.W., Besaw, L.C. and Aust, S.D. 1978. 2,4,5,3',4',5-hexabromobiphenyl is both a 3-methylcholanthrene- and a phenobarbital-type inducer of microsomal drug metabolizing enzymes. Biochem. Biophys. Res. Commun. 85: 450-458.

Ecobichon, D.J. and Comeau, A.M. 1974. Comparative effects of commercial Aroclors on rat liver enzyme activities. Chem. Biol. Interact. 9: 341-350.

Ecobichon, D.J. and Comeau, A.M. 1975. Isomerically pure chlorobiphenyl congeners and hepatic function in the rat: Influence of position and degree of chlorination. Toxicol. Appl. Pharmacol. 33: 94-105.

Ecobichon, D.J., Hansell, M.M. and Safe, S. 1977. Halogen substituents at the 4- and 4'- positions of biphenyl: Influence on hepatic function in the rat. Toxicol. Appl. Pharmacol. 42: 359-366.

Fujita, S., Tsuij, H., Kato, K., Saeki, S. and Tsukamoto, H. 1971. Effects of biphenyl chlorides on rat liver microsomes. Fukuoka Acta Med. 62: 30-34.

Goldstein, J.A. 1979. The structure-activity relationships of halogenated biphenyls as enzyme inducers. Ann. N.Y. Acad. Sci. 320: 164-178.

Goldstein, J.A., Hickman, P., Burse, V.W. and Bergman, H. 1975. A comparative study of two PCB mixtures (Aroclor 1242 and 1016) containing 42% chlorine on induction of hepatic porphyria and drug metabolizing enzymes. Toxicol. Appl. Pharmacol. 32: 461-473.

Goldstein, J.A., McKinney, J.D., Lucier, G.W., Hickman, P., Bergman, H. and Moore, J.A. 1976. Toxicological assessment of hexachlorobiphenyl isomers and 2,3,7,8-tetrachlorodibenzofuran in chicks. II. Effects on drug metabolism and porphyrin accumulation. Toxicol. Appl. Pharmacol. 36: 81-92.

Goldstein, J.A., Hickman, P., Bergman, H., McKinney, J.D. and Walker, M.P. 1977. Separation of pure polychlorinated biphenyl isomers into two types of inducers on the basis of induction of cytochrome P-450 or P-448. Chem. Biol. Interact. 17: 69-87.

Goldstein, J.A., Hass, J.R., Linko, P. and Harvan, D.J. 1978. 2,3,4,8-Tetrachlorodibenzofuran in a commercially available 99% pure polychlorinated biphenyl isomer identified as the inducer of hepatic cytochrome P-448 and aryl hydrocarbon hydroxylase in the rat. Drug Metab. Dispos. 6: 258-264.

Holdrinet, M.v.H., Braun, H.E., Frank, R., Stopps, G.J., Smout, M.S. and McWader, J.W. 1977. Organochlorine residues in human adipose tissue and milk from Ontario residents. Can. J. Public Health 68: 74-80.

Hutzinger, O., Safe, S. and Zitko, V. 1974. The chemistry of PCBs. CRC Press Cleveland, Ohio.

Jensen, S. and Sundström, G. 1974. Structures and levels of most chlorobiphenyls in two technical PCB products and in human adipose tissue. Ambio 3: 70-76.

Johnstone, G.J., Ecobichon, D.J. and Hutzinger, O. 1974. The influence of pure polychlorinated biphenyl compounds on hepatic function in the rat. Toxicol. Appl. Pharmacol. 28: 66-81.

Kawanishi, S., Mizutani, T. and Sano, S. 1978. Induction of porphyrin synthesis in chick embryo liver cell culture by synthetic polychlorobiphenyl isomers. Biochem. Biophys. Acta 540: 83-92.

Kohli, K.K., Gupta, B.N., Albro, P.W., Mukhtar, H. and McKinney, J.D. 1979a. Biochemical effects of pure isomers of hexachlorobiphenyl: Fatty livers and cell structure. Chem. Biol. Interact. 25: 139-156.

Kohli, K.K., Mukhtar, H., Bend, J.R., Albro, P.W. and McKinney, J.D. 1979b. Biochemical effects of pure isomers of hexachlorobiphenyl-hepatic microsomal epoxide hydrase and cytosolic glutathione S-transferase activities in the rat. Biochem. Pharmacol. 28: 1444-1446.

Kohli, K.K., Philpot, R.M., Albro, P.W. and McKinney, J.D. 1980. Induction of different species of cytochrome P-450 by coplanar and noncoplanar isomers of hexachlorobiphenyl. Life Sci. 26: 945-952.

Landrigan, P.J. 1980. General population exposure to environmental concentrations of halogenated biphenyls. In Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. Edited by R.D. Kimbrough. Elsevier North-Holland, Amsterdam. pp. 267-280.

McKinney, J.D. and Singh, P. 1981. Structure-activity relationships in halogenated biphenyls: Unifying for structural specificity. Chem. Biol. Interact. 33: 271-283.

McKinney, J.D., Chae, K., Gupta, B.N., Moore, J.A. and Goldstein, J.A. 1976. Toxicological assessment of hexachlorobiphenyl isomers and 2,3,7,8-tetrachlorodibenzofuran in chicks. Toxicol. Appl. Pharmacol. 36: 65-80.

Mes, J., Campbell, D.S., Robinson, R.N. and Davies, D.J.A. 1977. Polychlorinated biphenyl and organochlorine pesticide residues in adipose tissue of Canadians. Bull. Environ. Contam. Toxicol. 17: 196-203.

Moron, M., Sundström, G. and Wachtmeister, C.A. 1973. Polychlorinated biphenyls. Acta Chem. Scand. 27: 3121-3122.

Okey, A.B., Bondy, G.P., Mason, M.E., Kahl, G.F., Eisen, H.J., Guenthner, T.M. and Nebert, D.W. 1979. Regulatory gene product of the Ah locus. Characterization of the cytosolic inducer-receptor complex and evidence for its nuclear translocation. J. Biol. Chem. 254: 11636-11648.

Parkinson, A., Cockerline, R. and Safe, S. 1980a. Polychlorinated biphenyl isomers and congeners as inducers of both 3-methylcholanthrene- and phenobarbitone-type microsomal enzyme activity. Chem. Biol. Interact. 29: 277-289.

Parkinson, A., Cockerline, R. and Safe, S. 1980b. Induction of both 3-methylcholanthrene- and phenobarbitone-type microsomal enzyme activity by a single polychlorinated biphenyl isomer. Biochem. Pharmacol. 29: 259-262.

Parkinson, A., Copp, L. and Safe, S. 1980c. The utility of the microsomal 4-chlorobiphenyl hydroxylase enzyme assay in distinguishing between phenobarbitone- and 3-methylcholanthrene-induced microsomal monooxygenases. Anal. Biochem. 105: 65-73.

Parkinson, A., Robertson, L., Safe, L. and Safe, S. 1980d. Polychlorinated biphenyls as inducers of hepatic microsomal enzymes: Structure-activity rules. Chem. Biol. Interact. 30: 271-285.

Parkinson, A., Robertson, L. and Safe, S. 1980e. Hepatic microsomal enzyme induction by 2,2',3,3',4,4'- and 2,2',3',4,4',5-hexachlorobiphenyl. Life Sci. 27: 2333-2337.

Parkinson, A., Robertson, L.W. and Safe, S. 1980f. Reconstituted breast milk PCBs as potent inducers of aryl hydrocarbon hydroxylase. Biochem. Biophys. Res. Commun. 96: 882-889.

Parkinson, A., Robertson, L., Safe, L. and Safe, S. 1981. Polychlorinated biphenyls as inducers of hepatic microsomal enzymes: Effects of di-ortho substitution. Chem. Biol. Interact. 31: 1-12.

Poland, A. and Glover, E. 1977. Chlorinated biphenyl induction of aryl hydrocarbon hydroxylase activity: A study of structure-activity relationship. Mol. Pharmacol. 13: 924-938.

Poland, A. and Glover, E. 1980. 2,3,7,8-Tetrachlorodibenzo-p-dioxin: Segregation of toxicity with the Ah locus. Mol. Pharmacol. 17: 86-94.

Poland, A., Greenlee, W.F. and Kende, A.S. 1979. Studies on the mechanism of action of the chlorinated dibenzo-p-dioxins and related compounds. Ann. N.Y. Acad. Sci. 320: 214-230.

Sissons, D. and Welti, D. 1971. Structural identification of polychlorinated biphenyls in commercial mixtures by gas-liquid chromatography, nuclear magnetic resonance and mass spectrometry. J. Chromatogr. 60: 15-32.

Stondard, M.D. and Greig, J.G. 1976. Different patterns of hepatic microsomal enzyme activity produced by administration of pure hexachlorobiphenyl isomers and hexachlorobenzene. Chem. Biol. Interact. 15: 365-379.

Sundström, G., Hutzinger, O. and Safe, S. 1976. The metabolism of chlorobiphenyls - A review. Chemosphere 5: 267-298.

Watanabe, I., Yakushiji, T., Kuwabara, K., Yoshida, S., Maeda, K., Kashimoto, T., Koyama, K. and Kunita, N. 1979. Surveillance of the daily PCB intake from diet of Japanese women from 1972 through 1976. Arch. Environ. Contam. Toxicol. 8: 67-75.

Yamamoto, H., Yoshimura, H., Fujita, M. and Yamamoto, T. 1976. Metabolic and toxicologic evaluation of 2,3,4,3',4'-pentachlorobiphenyl in rats and mice. Chem. Pharm. Bull. 24: 2168-2174.

Yoshimura, H., Ozawa, N. and Saeki, S. 1978. Inductive effect of polychlorinated biphenyls mixture and individual isomers on the hepatic microsomal enzymes. Chem. Pharm. Bull. 26: 1216-1221.

Yoshimura, H., Yoshihara, S., Ozawa, N. and Miki, M. 1979. Possible correlation between induction modes of enzymes by PCBs and their toxicity in rats. Ann. N.Y. Acad. Sci. 320: 179-192.

Yoshimura, S., Kawano, K., Yoshimura, H., Kuroki, H. and Masuda, Y. 1979. Toxicological assessment of highly chlorinated biphenyl congeners retained in the Yusho patterns. Chemosphere 8: 531-538.

MIXED FUNCTION OXIDASES OF FISH LIVER AND MAMMALIAN LUNG: INTERACTIONS WITH XENOBIOTICS

Francis C.P. Law[a]

ABSTRACT

As a result of an increased awareness of environmental pollution, the mixed function oxidase (MFO) systems of mammalian lung and fish liver have received increasing attention in recent years. These MFO systems, like those of mammalian liver, are localized mainly in the endoplasmic reticulum of the cell and are composed of lipid, NADPH cytochrome P-450 reductase and cytochrome P-450. However, MFO activities in the mammalian lung and fish liver are much lower than that of the mammalian liver.

As with the mammalian liver, microsomes of the mammalian lung and fish liver contain at least two different forms of cytochrome P-450. Moreover, pulmonary and fish cytochrome P-450s possess properties that are characteristic of mammalian liver cytochrome P-448 (P_1-450). Thus, microsomal cytochrome P-450 contents and aryl hydrocarbon hydroxylase (AHH) activities in the mammalian lung and fish liver are significantly induced following pretreatment of animals with cytochrome P-448 inducers of mammalian liver: polycyclic aromatic hydrocarbons or polychlorinated biphenyls. Phenobarbital, a cytochrome P-450 inducer in mammalian liver, is not an effective inducer of MFO in fish liver or lung. Lung AHH has therefore been proposed as a short-term bioassay for cigarette toxicity, in contrast to fish hepatic AHH, which has been used as an index to determine whether fish have been previously exposed to crude oil or petroleum.

It is important to examine in detail the characteristics of the MFO systems of fish liver and the mammalian lung and the interactions of a single chemical with the various components of the MFO systems before investigating the combined effects of xenobiotics. Such studies will continue to provide insights into the complex effects of combined chemicals.

Beside subcellular fractions of mammalian lung and fish liver, the isolated perfused mammalian lung and the fish in vivo should also be used, respectively, to examine the combined effects of chemicals on the MFO systems of mammalian lung and fish liver.

[a] College of Pharmacy, Dalhousie University, Halifax, Nova Scotia B3H 4H6.

LES SYSTEMES D'OXYDASES A FONCTIONS MULTIPLES DANS LE FOIE CHEZ LES POISSONS ET DANS LES POUMONS CHEZ LES MAMMIFERES: INTERACTIONS AVEC LES XENOBIOTIQUES

Francis C.P. Law[a]

RESUME

Devant l'intérêt accru porté à la pollution de l'environnement, on s'est intéressé de plus en plus au cours des dernières années aux systèmes d'oxydases à fonctions multiples (OFM) dans le foie des poissons et dans les poumons des mammifères. Ces systèmes, tout comme ceux dans le foie des mammifères, se trouvent principalement dans le réticulum endoplasmique des cellules et sont constitués de lipides, du NADPH, de la cytochrome P-450 réductase et du cytochrome P-450. Toutefois, l'activité de ces enzymes dans les poumons des mammifères et dans le foie des poissons est beaucoup plus faible que dans le foie des mammifères.

Tout comme dans le foie des mammifères, les microsomes des poumons des mammifères et du foie des poissons renferment au moins deux formes différentes du cytochrome P-450. De plus, le cytochrome P-450 dans les poumons des mammifères et dans le foie des poissons présente des propriétés qui sont caractéristiques du cytochrome P-448 (P_1-450) du foie des mammifères. Ainsi, il y a accroissement de la teneur microsomique en cytochrome P-450 et induction importante de l'activité de l'aryl-hydrocarbure-hydroxylase (AHH) dans les poumons des mammifères et dans le foie des poissons après pré-traitement des animaux avec des inducteurs du cytochrome P-448 provenant du foie des mammifères, comme les hydrocarbures aromatiques polycycliques et les polychlorobiphényles. Le phénobarbital, qui est un inducteur du cytochrome P-450 du foie des mammifères, n'induit pas de façon efficace les oxydases à fonctions multiples dans le foie des poissons ou dans les poumons des mammifères. Par conséquent, on a proposé d'employer le taux de AHH dans les poumons comme épreuve à court terme pour déterminer la toxicité de la cigarette, au lieu du taux de AHH dans le foie des poissons, qui auparavant servait d'indice pour déterminer si un poisson avait préalablement été exposé à du mazout ou à du pétrole.

Il importe d'examiner en détail les caractéristiques des systèmes OFM dans le foie des poissons et les poumons des mammifères et d'étudier les interactions d'un seul produit avec divers composants de ces systèmes enzymatiques avant d'entreprendre l'étude de l'effet global des xénobiotiques. De telles études permettront d'approfondir encore plus l'effet complexe de plusieurs produits chimiques.

[a] College of Pharmacy, Dalhousie University, Halifax, Nouvelle Ecosse. B3H 4H6.

Outre des fractions subcellulaires de poumons, on devrait aussi utiliser des cellules pulmonaires isolées et des poumons isolés perfusés pour examiner l'effet de xénobiotiques sur les système OFM dans les poumons des mammifères et le foie des poissons.

INTRODUCTION

Mammalian lung and fish liver both occupy a unique and important position in the study of environmental toxicology. The lung is in direct contact with respirable airborne pollutants and other environmental agents. The extent of xenobiotic absorption and metabolism by the lung may significantly affect the toxicity of a chemical. Fishes are in direct contact with aquatic environment, which is also a storehouse for many environmental pollutants. The ability of aquatic species to metabolize foreign compounds is important not only for their own survival, but also for man who uses these species as food.

As a result of a growing awareness of environmental pollution caused by various pesticides and industrial organic chemicals, the mixed function oxidase (MFO) systems of mammalian lung and fish liver have received increasing attention in recent years. One of the urgent but unresolved problems of environmental pollution at present is the universal exposure of living organisms to levels not yet overtly toxic. Studies on the MFO systems in mammalian lung and fish liver may provide useful information on such sublethal effects.

Although our knowledge of the MFO system of the mammalian liver is substantial, our understanding of the MFO systems in the mammalian lung and fish liver is meager. The purpose of this presentation is to describe the uniqueness of mammalian lung and fish MFO systems. The interactions between the MFO systems and xenobiotics and the potential uses of these MFO systems as indicators of sublethal effects of environmental pollution will be examined.

THE MFO SYSTEM OF MAMMALIAN LUNG

CHARACTERISTICS OF LUNG MFO

Although the liver is the main metabolic site of foreign compounds, the lung is also capable of metabolizing a variety of xenobiotics. A list of chemicals that serve as substrates for the pulmonary MFO system can be found in the review articles of Brown (1974), and Gram (1980). Common phase I reactions in mammalian lungs include N- and O-demethylation (Bend et al. 1972; Ichikawa et al. 1969), aromatic hydroxylation (Bend et al. 1972; Bingham et al. 1976; Harper et al. 1973), aliphatic hydroxylation (Ichikawa et al. 1969), N-hydroxylation (Uehleke 1969; Uehleke 1971), N-oxidation (Uehleke 1969; Uehleke 1971; Devereux and Fouts 1974), epoxidation, and epoxide hydration (Grover et al. 1973; Grover et al. 1974; Grover 1974). Phase II reaction enzymes in mammalian lung include N-acetyltransferase, UDP-glucuronyl transferase and glutathione S-aryltransferase (Gram et al. 1974).

The MFO system of the rabbit lung has been extensively studied because it contains a high level of MFO activity (Oppelt et al. 1970). Comparison of rabbit lung and liver MFO systems reveals that, except for

minor differences, they are very similar. This conclusion is based on a comparison of the MFO systems of rabbit lung and liver with respect to: (a) the nature of the electron transfer system, (b) morphology of microsomes, (c) characteristics and subcellular distribution of MFO activity, and (d) multiple forms of cytochrome P-450.

The nature of the electron transfer systems in lung and liver are qualitatively similar. Thus, oxygen and NADPH are required for optimum MFO activity in lung and liver microsomes of rabbits (Matsubara et al. 1975; Bend et al. 1972).

Electron micrographs of resuspended liver or lung microsomal pellets of rabbits showed that rough and smooth endoplasmic reticulum vesicles, free ribosomes and particulate glycogen are the main elements. However, lung microsomal vesicles are often clumped or aggregated together (Bend et al. 1972).

In rabbit liver and lung subcellular fractions, the specific concentrations of microsomal marker enzymes such as aniline 4-hydroxylase, benzphetamine N-demethylase, biphenyl 4-hydroxylase, NADPH cytochrome c reductase, and cytochrome P-450 are highest in the microsomal fraction of both organs. Apparent Michaelis constants (K_m) do not differ appreciably between lung and liver for any of the substrates examined, although marked differences in V_{max} were observed (Bend et al. 1972; Gram 1973).

The laboratories of Philpot and co-workers (Serabjit-Singh et al. 1979; Wolf et al. 1978) and Guengerich (1977) have each isolated two forms of cytochrome P-450 from lung microsomes of untreated rabbits. The two forms of cytochrome P-450 are present in rabbit lung microsomes in equal amount (Franklin et al. 1980). Moreover, only one form of the pulmonary cytochrome P-450 is substantially induced following subcutaneous injection of 2,3,7,8-tetrachlorodibenzo-p-dioxin to rabbits (Liem et al. 1980).

INDUCTION OF PULMONARY MFO

The effect of pretreating animals with a single chemical on pulmonary MFO activities is summarized in Table 1. Phenobarbital, a good inducing agent for mammalian liver MFO, has little effect on the pulmonary system. In contrast, polycyclic aromatic hydrocarbons (3-methylcholanthrene and benzpyrene) are shown to be potent inducers of pulmonary MFO. Many natural and synthetic flavones, phenothiazines and substituted benzothiazoles are also inducers of pulmonary AHH.

Care must be exercised in extrapolating the effects of a single chemical on pulmonary MFO to that of multiple chemicals. For example, inhalation or intravenous administration of nickel carbonyl blocks the phenothiazine-induced AHH activity in rat lung. No inhibition of activity, however, was seen when nickel carbonyl was added in vitro to

Table 1. Induction of pulmonary MFO activity after pretreatment of animals with a single chemical.

Inducer	Reactions studied	Results[a]	References
Phenobarbital	cytochrome P-450	-	Uehleke (1969); Oppelt et al. (1970); Litterst et al. (1977); Matsubara et al. (1974)
	cytochrome b_5	-	Matsubara et al. (1974)
	NADPH cytochrome c reductase	-	Litterst et al. (1977)
	methylaniline-N-demethylase	-	Uehleke (1969)
	aminopyrine-N-demethylase	-	Oppelt et al. (1970)
	methylaniline hydroxylase	-	Uehleke (1969)
	aniline hydroxylase	-	Oppelt et al. (1970)
	bromobenzene hydroxylase	-	Reid et al. (1973)
	toluene hydroxylase	-	Carlone and Fouts (1974)
	biphenyl hydroxylase	-	Litterst et al. (1977)
	AHH	-	Matsubara et al. (1974); Wiebel et al. (1971)
	parathion (phosphorothionate) oxidation	+	Poore and Neal (1972)
3-Methylcholanthrene	cytochrome P-450	+	Matsubara et al. (1974); Litterst et al. (1977)
	cytochrome b_5	+	Wiebel et al. (1971); Matsubara et al. (1974)
	NADPH cytochrome c reductase	+	Capdevila et al. (1975)
	Δ^1-tetrahydrocannabinol	+	Nakazawa and Costa (1971); Law and So (1981)
	AHH	+	Gelboin and Blackburn (1964); Matsubara et al. (1974); Bingham et al. (1976); Gilman and Conney (1963)

Table 1. (cont'd).

Benzpyrene (Intratracheal instillation)	AHH	+	Okamoto et al. (1972); Mitchell (1980)
Phenothiazines	AHH	+	Wattenberg et al. (1968a)
Flavones	AHH	+	Wattenberg et al. (1968b)
Benzothiazoles	AHH	+	Wattenberg et al. (1968c)

[a] -, not inducible; +, inducible

phenothiazine-induced rat lung homogenate (Sunderman 1967). The effects of multiple chemicals on pulmonary MFO activities are snown in Table 2. Tobacco smoke, cannabis and diesel emission have been shown to enhance pulmonary AHH activity significantly. In contrast, fumes of heated rosin (Colophony), which are a mixture of natural products from pine trees, decrease pulmonary ethoxycoumarin O-deethylase activity of the rat (Zitting 1980). The effect of cigarette smoke on mammalian lung AHH activity appears to be species-specific. Thus, in contrast to the results of rodents and humans, pulmonary AHH activities of rabbits (Lubawy and Perrier 1980) and guinea pigs (Bilimoria et al. 1977) are severely depressed after acute exposure of animals to tobacco smoke.

The above studies demonstrate that polycyclic aromatic hydrocarbons are the most potent inducers of one of the cytochrome P-450s in the lung. The readily inducible form of cytochrome P-450 in the lung is responsible for the increased AHH activity following inhalation of cigarette smoke. Thus, development of lung cancer in heavy smokers might depend on the inducibility of this form of cytochrome P-450 in the lung (Remmer 1975). The finding that polycyclic hydrocarbons are present in a tumorigenic fraction of cigarette smoke condensate and are responsible for most of the AHH induction observed in animal tissue (Akin et al. 1975; Kouri et al. 1975) further supports this hypothesis.

Since pulmonary AHH activity in rodents is readily induced by cigarette smoke and since AHH induction is dose-dependent, lung AHH has been suggested as a short-term bioassay to determine cigarette toxicity (Akin and Benner 1976; Bilimoria and Ecobichon 1980).

PROBLEMS RELATED TO THE COMBINED EFFECTS OF XENOBIOTICS

As shown in Tables 1 and 2, the MFO system of rat lung is readily enhanced after pretreatment of animals with a variety of chemicals. However, the pulmonary MFO of rabbits appears to be less responsive to chemical inducers (Carlone and Fouts 1974; Uehleke 1971). Hence, with the exception of the pulmonary metabolism of Δ^1-tetrahydrocannabinol (Law and So 1981) and benzpyrene (Hulshoff et al. 1978; Bingham et al. 1976; Lubawy and Isaac 1980), numerous attempts to induce the metabolism of xenobiotics by rabbit lungs have been unsuccessful (Carlone and Fouts 1974; Uehleke 1971).

Mammalian lungs have more than 40 different cell types (Sorokin 1970). The isolation of a homogeneous population of lung cells which contain a high level of MFO activity is a first step toward understanding the complex events leading to pulmonary toxicity. Recently, Devereux and associates (1979, 1980) isolated viable cells from rabbit lungs and demonstrated that the alveolar type II cell and the bronchiolar Clara cell (nonciliated bronchiolar epithelial cell) contain the largest proportions of pulmonary endoplasmic reticulum. Similarly, using an autoradiographic technique, Boyd (1977) showed that the Clara cell may be a site of pulmonary MFO activity.

Table 2. Induction of pulmonary MFO activities following pretreatment of animals with a mixture of chemicals.

Chemical mixtures	Reactions studied	Species	Results[α]	References
Tobacco smoke	AHH	rat	+	Welch et al. (1971); Welch et al. (1972); Van Cantford and Gielen (1975); Van Cantford and Gielen (1977)
		mouse	+	Abramson and Hutton (1975); Holt and Keast (1973); Van Cantfort and Gielen (1975)
		guinea pig	-	Bilimoria et al. (1977)
		rabbit	-	Lubawy and Perrier (1980)
		human	+	Cantrell et al. (1973)
Cannabis	AHH	rat	+	Witschi and Saint-Francois (1972); Marcotte and Witschi (1972).
Diesel emission	AHH	rat	+	Lee et al. (1980)
Fumes of heated rosin	ethoxycoumarin-O-deethylase	rat	-	Zitting (1980)

[α] +, induction; -, inhibition

A question that still remains to be settled is to what extent the combined effects of xenobiotics (which are clearly demonstrable in the subcellular fractions of the lung) can be extrapolated to the whole organ or in vivo. In general, preparation difference is not an important problem of xenobiotic metabolism by the lung. Thus, the rate of phencyclidine metabolism by rabbit lung microsomes was shown to be comparable to that of the isolated, perfused lung (IPL) (Law 1981). However, lung subcellular fractions may yield results different to that of the IPL for a number of chemicals (Law et al. 1974; Law 1978; Law and So 1981).

A POSSIBLE FUTURE DEVELOPMENT: COMBINED EFFECTS OF CHEMICALS ON THE MFO OF THE IPL

The advantages of using the IPL preparation to study the combined effect of xenobiotics on the lung are: (a) the cellular structures of the lung remain intact; hence, the processes of uptake, disposition, metabolism and elimination of xenobiotics by the lung can be studied simultaneously, (b) the lung is completely isolated from the animal and is not complicated by indirect effects from the rest of the animal body, (c) the lung is ventilated and perfused under conditions closely resembling those that occur in living animals (Law 1978), (d) chemicals can be administered to the lung by the intravascular or intratracheal route, and (e) the left and right lungs can be separated, ventilated and perfused simultaneously and independently (Tucker and Shertzer 1980); thus, the combined effect of xenobiotics on the lung may be studied with one of the lungs, using the other lung as a control. Table 3 shows a list of chemicals metabolized by the IPL.

Itakura et al. (1977) reported that pretreatment of rabbits with chlorpromazine increased p-nitroanisole O-demethylation by the IPL but phenobarbital pretreatment had no effect. Pretreatment of rabbits with 3-methylcholanthrene was shown to enhance significantly Δ^1-tetrahydrocannabinol metabolism by the IPL (Law and So 1981). Benzpyrene metabolism by the IPL also was found to increase significantly after pretreatment of rabbits with 3-methylcholanthrene (Bingham et al. 1976; Lubawy and Isaac 1980) or benzpyrene (Warshawsky et al. 1978). In contrast, Smith et al. (1978) did not observe a significant increase in benzpyrene metabolism by the IPL following pretreatment of rabbits with 3-methylcholanthrene.

The combined effects of chemicals on the IPL often are monitored by the rate of disappearance of a chemical from the perfusate or the rates of appearance of metabolites in the perfusate. Thus, the metabolism of benzpyrene by the IPL prepared from 3-methylcholanthrene-pretreated rats was shown to be inhibited by adding α-naphthoflavone, diethylmaleate, salicylamide or D-saccharo-1,4-lactone directly to the perfusate (Vähäkangas et al. 1979). The rate of glucuronide conjugation of 4-methylumbelliferone by the IPL of rats was increased following exposure of animals to cigarette smoke daily for 10 consecutive days

Table 3. Drugs and chemicals metabolized by the isolated perfused lung preparation.

Substrates	Species	References
A. Phase I reactions		
N-Dealkylation		
methadone	rabbit	Law et al. (1974)
N-methylaniline	cat	Kiese and Uehleke (1961)
nortriptyline	dog	Whitnack et al. (1972)
aminopyrine	rabbit	Armstrong et al. (1973)
p-chloro-N-methylaniline	rabbit	Armstrong et al. (1973)
Aromatic Hydroxylation		
benzpyrene	rabbit	Bingham et al. (1976)
O-Dealkylation		
p-nitroanisole	rat	Itakura et al. (1977)
Alkyl Hydroxylation		
pentobarbital	rabbit	Law et al. (1974)
phencyclidine	rabbit	Law and Farquharson (1980)
Δ^1-tetrahydrocannabinol	rabbit; rat	Law (1978); Law and So (1981)
B. Phase II (synthesis) reactions		
O-Methylation		
isoproterenol	dog	Briant et al. (1973)
isoetharine	dog	Briant et al. (1973)
Glucuronidation		
4-methylumbelliferone	rat	Aitio et al. (1976)
Glutathione Conjugation		
styrene oxide	rabbit	Ryan et al. (1976)

(Uotila et al. 1977). More recently, Lubawy and Perrier (1980) reported that the rate of benzpyrene metabolism by the IPL of rabbits was decreased when the IPL was exposed directly to cigarette smoke. They suggested that cigarette smoke had an immediate and a delayed effect on benzpyrene metabolism by the lung. The immediate and the delayed effects are, respectively, inhibition and enhancement of benzpyrene metabolism by the lung.

THE MFO SYSTEM OF FISH LIVER

CHARACTERISTICS OF FISH LIVER MFO SYSTEM

Most freshwater and marine fish can carry out many of the reactions used by the mammalian species to metabolize foreign compounds. C-Hydroxylation, O-dealkylation, N-oxidation, S-oxidation, epoxidation, epoxide hydration and desulfuration are common phase I pathways of xenobiotic metabolism found in fish (Sieber and Adamson 1977; Buhler and Rasmusson 1968) while conjugation with glucuronic acid, glycine, acetate, taurine and sulfate are common phase II reactions (Sieber and Adamson 1977; Lech and Bend 1980).

In comparison with the mammalian liver MFO system, foreign compound metabolism by the fish is also carried out mainly by the hepatic microsomal MFO system, which requires oxygen and NADPH for maximal activity. The temperature optimum of the fish MFO activities in vitro usually is 25°C, which is lower than the 37°C temperature optimum of the mammalian system (Buhler and Rasmusson 1968). Electron micrographs of resuspended little skate liver or lobster hepatopancreas demonstrate the presence of rough and smooth vesicles on which the MFO is believed to reside (Pohl et al. 1974). Like the mammalian liver, fish liver microsomes are composed of lipid, NADPH cytochrome P-450 reductase and cytochrome P-450 (Bend et al. 1977b). However, fish liver appears to have approximately 1/10 of the MFO activity of mammalian liver. Hence, levels of cytochrome P-450 of various marine fish are much lower than those seen in preparations from mammals (Pohl et al. 1974; Ahokas 1979).

Ahokas and co-workers (1977) reported that the cytochrome P-450 of trout liver microsomes appeared to possess the characteristics of mammalian liver P-450 and P-448. Thus, substances known to strongly inhibit the mammalian cytochrome P-450 were not very effective in trout cytochrome P-450. However, substances that are mammalian cytochrome P-448 inhibitors were very effective inhibitors of trout cytochrome P-450. The multiplicity of fish liver cytochrome P-450 has since been confirmed by Bend and co-workers (1979). They partially purified two forms of cytochrome P-450 from solubilized liver microsomes of 1,2,3,4-dibenzanthracene-pretreated little skate (Raja erinacea). One of the forms has its absorption maximum at 448 nm in the reduced and CO-ligated state and accounts for 50-60% of the total cytochrome P-450 in the 1,2,3,4-dibenzanthracene-induced fish. The other major form has an

absorption maximum at 451 nm and is very similar to the major form of hepatic cytochrome P-450 in untreated skate.

INDUCTION OF FISH MFO

Induction by a Single Chemical

Buhler and Rasmussen (1968) reported that feeding or injecting fish with technical DDT failed to induce the hepatic MFO system. This observation was confirmed by Addison and associates (1977) who fed trout (Salvelinus fontinalis) with DDT or DDE over a 4-wk period and did not detect any significant changes in the hepatic cytochrome P-450 content, aldrin epoxidase or 7-ethoxycoumarin O-deethylase activities. Similarly, Pohl and co-workers (1974) were unable to detect an increase in hepatic benzphetamine demethylase or benzpyrene hydroxylase activity after mummichog (Fundulus heteroclitus) were exposed to 0.1 mg/L DDT in sea water for 24 h and then left in DDT-free water for 4 days.

Addison and co-workers (1979) showed that trout liver cytochrome P-450 level was significantly induced following oral administration of butylated monochlorodiphenyl ethers (formerly Dow Chemical Co. XFS-4169L, now sold as Dielectric Fluid C4). However, aniline hydroxylase, aldrin epoxidase and umbelliferone O-deethylase activities were not altered. Trout liver MFO activities also did not change significantly after fish were orally fed other chlorinated diphenyl ethers, such as 4-chloro-, 2,4,4'-trichloro- or 2,4,5-trichlorodiphenyl ether (Chui et al. 1980).

Gruger and associates (1976) showed evidence of AHH induction by 2,4,2',4',5'-pentachlorobiphenyl in coho salmon (Oncorhynchus kisutch). Recently, Förlin and Lidman (1978) compared the effects of 4-chloro-, 2,5,2',5'-tetrachloro- and 2,4,5,2',4',5'-hexachlorobiphenyl on hepatic MFO activities in the rainbow trout (Salmo gairdneri) following a single intraperitoneal dose; 2,4,5,2',4',5'-hexachlorobiphenyl was found to enhance p-nitroanisole O-demethylation and microsomal cytochrome P-450 content. None of the chemicals affected NADPH cytochrome c reductase activity or the microsomal protein and phospholipid contents. Thus, the inductive capability of different specific chlorobiphenyls seems to depend on the content and arrangement of the chlorine atoms on the biphenyl molecules.

Pohl and associates (1976) reported that microsomal AHH activity was significantly enhanced following intraperitoneal administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to the little skate (Raja erinacea). Aniline hydroxylase and 7-ethoxycoumarin O-deethylase activities were also increased, but benzphetamine N-demethylase activity was not altered. Similar induction in AHH activity was observed in the winter flounder (Pseudopleuronetes americanus) after oral TCDD administration. Hepatic microsomal epoxide hydrase or glutathione S-transferase activities were not altered by the pretreatment.

Burns (1976) reported induction of aldrin epoxidation activity and cytochrome P-450 levels in the liver of an estuarine fish, Fundulus heteroclitus, maintained in seawater containing phenylbutazone. It is interesting to note that dietary exposure of rainbow trout to phenylbutazone also induced proliferation of smooth endoplasmic reticulum, the site of xenobiotic metabolism (Scarpelli 1977).

Exposure of fish to phenobarbital failed to elevate any MFO activity tested or cytochrome P-450 content (Buhler and Rasmussen 1968; Bend et al. 1977b; Förlin 1980; Hansson et al. 1980).

Hepatic microsomal AHH activity was shown to increase significantly following pretreatment of rainbow trout with 3-methylcholanthrene (Pedersen et al. 1974). The hepatic MFO activities of a variety of fish including rainbow trout (Gerhart and Carlson 1978; Statham et al. 1978; Elcombe and Lech 1979), little skate, sheepshead (Bend et al. 1977a) and northern pike (Balk et al. 1980) as well as salamander (Busbee et al. 1975) were also enhanced after exposure to polycyclic aromatic hydrocarbons.

Combined Effects of Two or More Chemicals

Combined Effects of Polyhalogenated Biphenyls

Following oral feeding or intraperitoneal administration of Clophen A50 (a commercial mixture of polychlorinated biphenyls produced by Bayer Chemicals, Germany) to rainbow trout (Salmo gairdneri), hepatic microsomal P-450 contents and p-nitroanisole O-demethylase and AHH activities increased significantly (Lidman et al. 1976; Förlin and Lidman 1978; Förlin 1980). Similarly, AHH, ethoxycoumarin and ethoxyresorufin-O-deethylases activities were markedly enhanced after the rainbow trout were pretreated intraperitoneally with Aroclor 1254, Aroclor 1242 or Fire Master BP-6 (150 mg/kg), although hepatic cytochrome P-450 content and ethylmorphine N-demethylation were not significantly increased (Elcombe and Lech 1978). Moreover, the ethoxycoumarin and ethoxyresorufin O-deethylases activities remained induced for at least two weeks after polyhalogenated biphenyl pretreatment. It is interesting to note that the AHH activity in embryos of an estuarine fish, Fundulus heteroclitus, was induced 3-fold after being exposed to 200 µg/L Aroclor 1254 in seawater (Binder and Stegeman 1980).

Following exposure of chlorinated biphenyls to channel catfish (Ictaluru punctatus) (Hinton et al. 1978; Klaunig et al. 1979), chinook salmon (Oncorhynchus tshawytscha) (Hawkes 1980), or rainbow trout (Salmo gairdneri) (Hacking et al. 1978), proliferation of endoplasmic reticulum and "bizarre" whorls of both the smooth and rough endoplasmic reticulum were seen in the liver of the fish. It has been suggested that such changes in the endoplasmic reticulum, particularly in the circular arrays of smooth endoplasmic reticulum, are responses to substances that induce the hepatic MFO system (Klaunig et al. 1979).

Combined Effects of Crude Oil or Petroleum

Crude oils contain polycyclic aromatic hydrocarbons which are known to be potent inducers of fish liver microsomal oxidases (see pg 190). Hence, the presence of inducible AHH in fish may provide a convenient means of assessing previous exposure to petroleum or crude oil.

In laboratory studies, Payne and Penrose (1975) showed that hepatic AHH activities of brown trout (Salmo trutta) and capelins (Mallotus villosus) were greatly increased after daily exposures to an emulsion of Tia Juana Medium Venezuela crude oil for two weeks. Hepatic AHH activities have also been shown to increase after coho salmon were exposed to 0.15 mg/L of a seawater-soluble fraction of Prudhoe Bay crude oil (Gruger et al. 1977). Fish liver size was increased by 50% after juvenile striped mullet (Mugil cephalus) were exposed for 4 days to emulsified Empire Mix or Saudi Arabian crude oil at an initial concentration of 75 mg/L (Yarbrough and Chambers 1977). Moreover, both hepatic cytochromes P-450 and b_5 were increased following oil treatment. Enhancement of AHH activity was also shown in Blennius pavo, a marine fish with a restricted territorial range, after the fish was exposed to either 170 µg/L diesel 2 oil for 14 days or water saturated with the oil for 3 days (Kurelec et al. 1977). The AHH activity was elevated for at least a month. In contrast, Microcosomes sulcatus, a benthic protochordate, showed no change in hepatic AHH activity even after 30 days of exposure (Kurelec et al. 1977). In a recent study, Payne and May (1979) demonstrated that exposure of cunners (Tautogolabrus adspersus) to 1-3 mL of Venezuelan crude as a surface slick in a 2-litre aquarium for 3 days induced not only hepatic AHH activity but also AHH activities in the heart, kidney, gills and spleen of the fish. However, the two polyethoxylate dispersant mixtures-- BP 1100X (BP Trading Ltd) and Oilsperse 43 (Diachem of B.C. Ltd.)-- had little effect in enhancing liver AHH when cunners were exposed to a mixture of oil and oil-spill dispersant. Oil dispersion does not appear to make the inducing agents more available to the fish.

In field studies, Payne and Penrose (1975) found that brown trout and capelins taken from waters heavily contaminated with oil and other pollutants had higher levels of AHH activity than fish collected from non-polluted waters. They suggested that inducible AHH could be used in fish to assess previous exposure to petroleum pollutants in water.

Similarly, Stegeman (1978) reported higher levels of AHH activity and cytochrome P-450 content in the liver of killifish (Fundulus heteroclitus) from Wild Harbor Marsh, the site of a 1969 oil spill, than in fish from "uncontaminated" marshes. The elevated enzyme activities were presumed to be related to the oil spill that occurred 8 years before the enzyme assays.

Kurelec et al. (1977) also demonstrated that sardines (Blennius pavo) caught at different locations had different, low levels of AHH

activities. In contrast, fish from the same location had similar levels of activity. They monitored the AHH activity in the livers of Blenniidae at different times following an oil pollution incident. Hepatic AHH activity reached a peak on the 23rd day and decreased to a new level (3 times the original pre-exposure level) on the 45th day. This new AHH level had not abated even 5 months after the incident.

When the hepatic ultrastructures of killifish from Wild Harbor Marsh were examined, an unusual increase in the amount of rough endoplasmic reticulum in the cell was found (Sabo and Stegeman 1977). Similarly, a dramatic increase in rough endoplasmic reticulum in liver cells of rainbow trout was also observed after the trout were exposed to high dietary doses of Prudhoe Bay crude oil in the laboratory for 8 months (Hawkes 1977).

IMPLICATIONS AND PROBLEMS OF FISH LIVER MFO INDUCTION STUDIES

Fish species accumulate environmental pollutants from water and can be expected to show MFO activity changes following chronic exposure to very low concentrations of these chemicals. Since the basal MFO activity of the fish population in the wild may have been modified by environmental pollutants, one good approach is to compare hepatic MFO properties in control and induced fish following their exposure to MFO activators. Hence, exposure of fish to environmental pollutants under rigidly controlled laboratory conditions will continue to provide insights into the complicated effects of combined xenobiotics on fish liver MFO.

From a toxicological viewpoint, AHH and ethoxycoumarin O-deethylase activities appear to be better indices than cytochrome P-450 content, NADPH cytochrome c reductase activity, microsomal protein, and phospholipid content for detecting previous exposure of fish to environmental pollutants. Thus, when Aroclor 5460, Fire Master BP-6 (polybrominated biphenyls) and other known or potential environmental contaminants were orally administered to brook trout (Salvelinus fontinalis), hepatic AHH hydroxylase and ethoxycoumarin O-deethylase activities were significantly elevated while fish weight, liver weight, microsomal protein and microsomal cytochrome P-450 contents were apparently unaffected (Law and Addison 1981). The hepatic AHH activity of fish has been used successfully to monitor water pollutants containing crude oil or polycyclic aromatic hydrocarbons. Moreover, hepatic ethoxycoumarin O-deethylation activity and the proliferation of endoplasmic reticulum in the liver cells show considerable promise as indices of previous exposure to polyhalogenated biphenyls and have generated much interest. Further studies, however, are needed to establish the usefulness of the proliferation of fish liver endoplasmic reticulum as a specific index of environmental pollution since Couch (1975) has concluded in his review that the effects of pesticides and related chemicals on the histopathology of fish liver generally are nonspecific.

POSSIBLE FUTURE DEVELOPMENTS

A Modified Ames Test

Kurelec and associates (1979) proposed to detect carcinogenic and mutagenic chemicals in water, respectively, with AHH and Salmonella tests (Ames 1972). They showed that when environmental pollutants were extracted from water by hexane and administered intraperitoneally to carp, hepatic AHH activity of the fish was increased significantly. In addition, the hexane extract gave positive results to a modified Ames test using fish liver post-mitochondrial supernatant. These biochemical tests, thus, provide a means to assess the potential carcinogenicity and mutagenicity of water pollutants without having to isolate and characterize each one of these chemicals in the sample. These assays are useful prescreen tests before long-term and expensive assays are planned. Additional studies with these biochemical tests should be pursued.

Pharmacokinetic Studies in Fish

Pharmacokinetic studies (i.e. the kinetics of absorption, distribution, biotransformation and elimination) with the fish *in vivo* provide another means to examine the effects of multiple chemicals on fish liver MFO. Since the plasma half-life of a chemical often reflects the hepatic MFO activity of an animal, the combined effects of chemicals on fish may be monitored by plasma half-life determinations. Moreover, these studies often permit the quantitative establishment of relationships between exposure level and toxic response. Pharmacokinetic studies require knowledge on the time course of chemical concentrations in blood and urine of the fish. At present, dogfish shark (Bungay *et al.* 1976), skate (Darrow *et al.* 1978) and flounder (Pritchard *et al.* 1973) appear to be the satisfactory fish models for these purposes, since these fish are large enough for serial samplings of blood and urine and the surgical techniques of placing cannuli in the fish are available.

SUMMARY

In view of the fact that living organisms are exposed chronically to low levels of environmental pollutants, it is important to develop assays which can monitor the presence as well as the biological effects of pollutants on living organisms. Although chemical analysis has been used successfully to detect very low levels of pollutants in the environment, it does not provide information on the environmental impact of pollutants on living organisms. The interactions between xenobiotics and the MFO systems, however, provide a means to monitor these effects.

The MFO systems of fish liver and mammalian lung possess mammalian liver cytochrome P-448 and P-450 characteristics and interact easily with polycyclic aromatic hydrocarbons and polychlorinated biphenyls. Many bioassays have been developed using the unique

properties of cytochrome P-448. These bioassays include: (a) the pulmonary AHH of rodents as a short-term bioassay to determine cigarette toxicity on mammalian lungs, (b) the induction of fish liver AHH as an index of previous exposure of fish to pollutants containing polycyclic aromatic hydrocarbons, and (c) the proposed use of a combination of AHH and Ames tests with fish liver subcellular fractions to detect potential carcinogenic and mutagenic chemicals in water. Additional studies, however, are still needed to firmly establish the usefulness of the MFO systems of fish liver and mammalian lung as indices of sublethal stress of environmental pollutants on living organisms.

Pharmacokinetic studies with the IPL and the fish *in vivo* on environmental pollutants also appear to be a good approach to examine the combined effects of xenobiotics on intact fish liver and mammalian lungs. Pharmacokinetic studies permit the quantitative establishment of the relationship between exposure levels and toxic response and allow the development of mathematical models which may be useful to predict the environmental impact of pollutants of similar chemical structures.

REFERENCES

Abramson, R.K. and Hutton, J.J. 1975. Effects of cigarette smoking on aryl hydrocarbon hydroxylase activity in lungs and tissues of inbred mice. Cancer Res. 35: 23-29.

Addison, R.F., Zinck, M.E. and Willis, D.E. 1977. Mixed function oxidase enzymes in trout (Salvelinus fontinalis) liver: absence of induction following feeding of p,p'-DDT or p,p'-DDE. Comp. Biochem. Physiol. 57: 39-43.

Addison, R.F., Zinck, M.E., Willis, D.E. and Darrow, D.C. 1979. Induction of hepatic mixed function oxidases in trout by polychlorinated biphenyls and butylated monochlorodiphenyl ethers. Toxicol. Appl. Pharmacol. 49: 245-248.

Ahokas, J.T. 1979. Cytochrome P-450 in fish liver microsomes and carcinogen activation. In Pesticide and xenobiotic metabolism in aquatic organisms. Edited by M.A.Q. Khan, J.J. Lech and J.J. Menn. American Chemical Society, Washington, D.C. pp. 279-296.

Ahokas, J.T., Pelkonen, O. and Kärki, N.T. 1977. Characterization of benzo[a]pyrene hydroxylase of trout liver. Cancer Res. 37: 3737-3743.

Aitio, A., Hartiala, J. and Uotila, P. 1976. Glucuronide synthesis in the isolated perfused rat lung. Biochem. Pharmacol. 25: 1919-1920.

Akin, F.J. and Benner, J.F. 1976. Induction of aryl hydrocarbon hydroxylase in rodent lung by cigarette smoke: a potential short-term bioassay. Toxicol. Appl. Pharmacol. 36: 331-337.

Akin, F.J., Chamberlain, W.J. and Chortyk, O.T. 1975. Mouse skin tumorigenesis and induction of aryl hydrocarbon hydroxylase by tobacco smoke fractions. J. Natl. Cancer Inst. 54: 907-912.

Ames, B.N. 1972. A bacterial system for detecting mutagens and carcinogens. In Mutagenic effects of environmental contaminants. Edited by H.E. Sutton and M.I. Harris. Academic Press, New York. pp. 57-66.

Armstrong, V.L., Niemeier, R.W. and Bingham, E. 1973. N-Demethylation of aminopyrine and p-chloro-N-methylaniline by isolated perfused rabbit lung. Toxicol. Appl. Pharmacol. 25: 491.

Balk, L., Meijer, J., Seidegard, J., Morgenstein, R. and DePierre, J.W. 1980. Initial characterization of drug-metabolizing systems in the liver of the northern pike, Esox lucius. Drug Metab. Dispos. 8: 98-103.

Bend, J.R., Hook, G.E.R., Easterling, R.E., Gram, T.E. and Fouts, J.R. 1972. A comparative study of the hepatic and pulmonary microsomal mixed-function oxidase systems in the rabbit. J. Pharmacol. Exp. Ther. 183: 206-217.

Bend, J.R., James, M.O. and Dansette, P.M. 1977a. In vitro metabolism of xenobiotics in some marine animals. Ann. N.Y. Acad. Sci. 298: 505-521.

Bend, J.R., Pohl, R.J., Arinc, E. and Philpot, R.M. 1977b. Hepatic microsomal and solubilized mixed-function oxidase systems from little skate, Raja erinacea, a marine elasmobranch. In Microsomes and drug oxidation. Edited by V. Ullrich, I. Roots, A. Hildebrandt, R.W. Estabrook and A.H. Conney. Pergamon Press, Oxford. pp. 160-169.

Bend, J.R., Ball, L.M., Elmamlouk, T.H., James, M.O. and Philpot, R.M. 1979. Microsomal mixed-function oxidation in untreated and polycyclic aromatic hydrocarbon-treated marine fish. In Pesticide and xenobiotic metabolism in aquatic organisms. Edited by M.A.Q. Khan, J.J. Lech and J.J. Menn. American Chemical Society, Washington, D.C. pp. 297-318.

Bilimoria, M.H. and Ecobichon, D.J. 1980. Responses of rodent hepatic, renal and pulmonary aryl hydrocarbon hydroxylase following exposure to cigarette smoke. Toxicology 15: 83-89.

Bilimoria, M.H., Johnson, J., Hogg, J.C. and Witschi, H.P. 1977. Pulmonary aryl hydrocarbon hydroxylase: tobacco smoke-exposed guinea pigs. Toxicol. Appl. Pharmacol. 41: 433-440.

Binder, R.L. and Stegeman, J.J. 1980. Induction of aryl hydrocarbon hydroxylase activity in embryos of an estuarine fish. Biochem. Pharmacol. 29: 949-951.

Bingham, E., Niemeier, R. and Dalbey, W. 1976. Metabolism of environmental pollutants by the isolated perfused lung. Fed. Proc. 35: 81-84.

Boyd, M.R. 1977. Evidence for the Clara cell as a site of cytochrome P450-dependent mixed-function oxidase activity in lung. Nature 269: 713-715.

Briant, R.H., Blackwell, E.W., Williams, F.M., Davies, D.S. and Dollery, C.T. 1973. The metabolism of sympathomimetic bronchodilator drugs by the isolated perfused dog lung. Xenobiotica 3: 787-799.

Brown, E.A.B. 1974. The localization, metabolism and effects of drugs and toxicants in lung. Drug Metab. Rev. 3: 33-87.

Buhler, D.R. and Rasmusson, M.E. 1968. The oxidation of drug by fishes. Comp. Biochem. Physiol. 25: 223-239.

Bungay, P.M., Dedrick, R.B. and Guarino, A.M. 1976. Pharmacokinetic modeling of the dogfish shark (Squalus acanthias): distribution and urinary and biliary excretion of phenol red and its glucuronide. J. Pharmacokinet. Biopharm. 4: 377-388.

Burns, K.A. 1976. Microsomal mixed function oxidases in an estuarine fish, Fundulus heteroclitus, and their induction as a result of environmental contamination. Comp. Biochem. Physiol. 53B: 443-446.

Busbee, D., Colvin, D., Muijsson, I., Rose, F. and Cantrell, E. 1975. Induction of aryl hydrocarbon hydroxylase in Ambystoma tigrinum. Comp. Biochem. Physiol. 50C: 33-36.

Cantrell, E.T., Warr, G.A., Busbee, D.L. and Martin, R.R. 1973. Induction of aryl hydrocarbon hydroxylase in human alveolar macrophages by cigarette smoking. J. Clin. Invest. 52: 1881-1884.

Capdevila, J., Jakobsson, S.W., Jernström, B., Helia, O. and Orrenius, S. 1975. Characterization of a rat lung microsomal fraction obtained by Sepharose 2B ultrafiltration. Cancer Res. 35: 2820-2829.

Carlone, M.F. and Fouts, J.R. 1974. In vitro metabolism of p-xylene by rabbit lung and liver. Xenobiotica 4: 705-715.

Chui, Y.C., Willis, D.E., Addison, R.F. and Law, F.C.P. 1980. Unpublished data. College of Pharmacy, Dalhousie University, Halifax, N.S.

Couch, J.A. 1975. Histopathological effects of pesticides and related chemicals on the liver of fishes. In The pathology of fish. Edited by W.E. Ribelin and G. Migaki. University of Wisconsin Press, Madison. pp. 559-584.

Darrow, D.C., Addison, R.F. and Law, F.C.P. 1978. Disposition of diphenyl ether following intravenous administration to thorny skate (Raja radiata). Comp. Biochem. Physiol. 61C: 317-321.

Devereux, T.R. and Fouts, J.R. 1974. N-oxidation and demethylation of N,N-dimethylaniline by rabbit liver and lung microsomes: effects of age and metals. Chem.-Biol. Interact. 8: 91-105.

Devereux, T.R. and Fouts, J.R. 1980. A procedure for isolation of rabbit pulmonary epithelial cells for study of foreign compound metabolism. In Microsomes, drug oxidation and chemical carcinogenesis. Edited by M.J. Coon, A.H. Conney, R.W. Estabrook, H.V. Gelboin, J.R. Gillette and P.J. O'Brien. Academic Press, New York. pp. 825-828.

Devereux, T.R., Hook, G.E.R. and Fouts, J.R. 1979. Foreign compound metabolism by isolated cells from rabbit lung. Drug Metab. Dispos. 7: 70-75.

Elcombe, C.R. and Lech, J.J. 1978. Induction of monooxygenation in rainbow trout by polybrominated biphenyls: a comparative study. Environ. Health Perspect. 23: 309-314.

Elcombe, C.R. and Lech, J.J. 1979. Induction and characterization of hemoprotein(s) P-450 and monooxygenation in rainbow trout (Salmo gairdneri). Toxicol. Appl. Pharmacol. 49: 437-450.

Förlin, L. 1980. Effects of Clophen A50, 3-methylcholanthrene, pregnenolone-16α-carbonitrile and phenobarbital on the hepatic microsomal cytochrome P-450-dependent monooxygenase system in rainbow trout, Salmo gairdneri, of different age and sex. Toxicol. Appl. Pharmacol. 54: 420-430.

Förlin, L. and Lidman, U. 1978. Effects of Clophen A50, 4- , 2,5,2',5'-tetra- and 2,4,5,2',4',5'-hexachlorobiphenyl on the mixed-function oxidase system of rainbow trout (Salmo gairdneri Rich) liver. Comp. Biochem. Physiol. 60C: 193-197.

Franklin, M.R., Wolf, C.R., Serabjit-Singh, C. and Philpot, R.M. 1980. Quantitation of two forms of pulmonary cytochrome P-450 in microsomes, using substrate specificities. Mol. Pharmacol. 17: 415-420.

Gelboin, H.V. and Blackburn, N.R. 1964. The stimulatory effect of 3-methylcholanthrene on benzpyrene hydroxylase activity in several rat tissues: inhibition by actinomycin D and puromycin. Cancer Res. 24: 356-360.

Gerhart, E.H. and Carlson, R.M. 1978. Hepatic mixed-function oxidase activity in rainbow trout exposed to several polycyclic aromatic compounds. Environ. Res. 17: 284-295.

Gilman, A.G. and Conney, A.H. 1963. The induction of aminoazo dye N-demethylase in nonhepatic tissues by 3-methylcholanthrene. Biochem. Pharmacol. 12: 591-593.

Gram, T.E. 1973. Comparative aspects of mixed-function oxidation by lung and liver of rabbits. Drug Metab. Rev. 2: 1-32.

Gram, T.E. 1980. The metabolism of xenobiotics by mammalian lung. In Extrahepatic metabolism of drugs and other foreign compounds. Edited by T.E. Gram. SP Medical and Scientific Books, New York. pp. 159-209.

Gram, T.E., Litterst, C.L. and Mimnaugh, E.G. 1974. Comparative aspects of drug metabolism by mammalian lung and liver: mixed function oxidation and conjugation reactions. In Drug interactions. Edited by P.L. Morselli, S. Garanttini and S.N. Cohen. Raven Press, New York. pp. 103-109.

Grover, P. 1974. K-regions of polycyclic hydrocarbons: formation and further metabolism by rat lung preparations. Biochem. Pharmacol. 23: 333-343.

Grover, P.L., Hewer, A. and Sims, P. 1973. K-region epoxides of polycyclic hydrocarbons: formation and further metabolism of benz[a]anthracene 5,6-oxide by human lung preparations. FEBS Lett. 34: 63-68.

Grover, P.L., Hewer, A. and Sims, P. 1974. Metabolism of polycyclic hydrocarbons by rat lung preparations. Biochem. Pharmacol. 23: 323-332.

Gruger, E.H. Jr., Hruby, T. and Karrick, N.L. 1976. Sublethal effects of structurally related tetrachloro-, pentachloro- and hexachlorobiphenyl on juvenile coho salmon. Environ. Sci. Technol. 10: 1033-1037.

Gruger, E.H. Jr., Wekell, M.M., Numoto, P.J. and Craddock, D.R. 1977. Induction of hepatic aryl hydrocarbon hydroxylase in salmon exposed to petroleum dissolved in seawater and to petroleum and polychlorinated biphenyls, separate and together, in food. Bull. Environ. Contamin. Toxicol. 17: 512-520.

Guengerich, F.P. 1977. Preparation and properties of highly purified cytochrome P-450 and NADPH cytochrome P-450 reductase from pulmonary microsomes of untreated rabbits. Mol. Pharmacol. 13: 911-923.

Hacking, M.A., Budd, J. and Hodson, K. 1978. The ultrastructure of the liver of the rainbow trout: normal structure and modification after chronic administration of a polychlorinated biphenyl Aroclor 1254. Can. J. Zool. 56: 477-491.

Hansson, T., Rafter, J. and Gustafsson, J.A. 1980. Effects of some common inducers on the hepatic microsomal metabolism of androstenedione in rainbow trout with special reference to cytochrome P-450-dependent enzymes. Biochem. Pharmacol. 29: 583-587.

Harper, C., Drew, R.T. and Fouts, J.R. 1973. Species differences in benzene hydroxylation to phenol by pulmonary and hepatic microsomes. Drug Metab. Dispos. 3: 381-388.

Hawkes, J.W. 1977. The effects of petroleum hydrocarbon exposure on the structure of fish tissues. In Fate and effects of petroleum hydrocarbons in marine ecosystems and organisms. Edited by D.A. Wolfe. Pergamon Press, New York. pp. 115-128

Hawkes, J.W. 1980. The effects of xenobiotics on fish tissues: morphological studies. Fed. Proc. 39: 3230-3236.

Hinton, D.E., Klaunig, J.E. and Lipsky, M.M. 1978. PCB-induced alterations in teleost liver: a model for environmental disease in fish. Mar. Fish. Rev. 4(10): 47-50.

Holt, P.G. and Keast, D. 1973. Induction of aryl hydrocarbon hydroxylase in the lungs of mice in response to cigarette smoke. Experientia 29: 1004.

Hulshoff, W.C., Lubawy, W.C. and Kostenbauder, H.B. 1978. The effect of some cigarette smoke constituents and other compounds on the metabolism of benzo(a)pyrene in rabbit lung 9000 g supernatant. Xenobiotica 8: 711-718.

Ichikawa, Y., Yamano, T. and Fujishima, H. 1969. Relationship between the interconversion of cytochrome P-450 and P-420 and its activities in hydroxylations and demethylations by P-450 oxidase systems. Biochim. Biophys. Acta 171: 32-46.

Itakura, N., Fisher, A.B. and Thurman, R.G. 1977. Cytochrome P-450 linked p-nitroanisole O-demethylation in the perfused lung. J. Appl. Physiol. 43: 238-245.

Kiese, M. and Uehleke, H. 1961. Der Ort der N-Oxydation des Aniline im höheren Tier. Naunyn-Schmiedeberg's Arch. Pharmacol. 242: 117-129.

Klaunig, J.E., Lipsky, M.M., Trump, B.F. and Hinton, D.E. 1979. Biochemical and ultrastructural changes in teleost liver following subacute exposure to PCB. J. Environ. Pathol. Toxicol. 2: 953-963.

Kouri, R., Whitmire, C. and Benedict, W. 1975. In vivo and in vitro effects of cigarette smoke condensate fractions. Proc. Am. Assoc. Cancer Res. 16: 173.

Kurelec, B., Britvic, S., Rijavec, M., Muller, W.E.G. and Zahn, R.K. 1977. Benzo(a)pyrene monooxygenase induction in marine fish -- molecular response to oil pollution. Mar. Biol. 44: 211-216.

Kurelec, B., Matijasevic, Z., Rijavec, M., Alacevic, M., Britvic, S., Muller, W.E.G. and Zahn, R.K. 1979. Induction of benzo(a) pyrene monooxygenase in fish and the Salmonella test as a tool for detecting mutagenic/carcinogenic xenobiotics in the aquatic environment. Bull. Environ. Contam. Toxicol. 21: 799-807.

Law, F.C.P. 1978. Metabolism and disposition of Δ^1-tetrahydrocannabinol by the isolated perfused rabbit lung. Drug Metab. Dispos. 6: 154-163.

Law, F.C.P. 1981. Phencyclidine metabolism by the isolated perfused lung of rabbits: comparison with lung and liver microsomes. Drug Metab. Dispos. (in press).

Law, F.C.P. and Addison, R.F. 1981. Response of trout hepatic mixed-function oxidases to experimental feeding of ten known or possible chlorinated environmental contaminants. Bull. Environ. Contam. Toxicol. 26: 243-247.

Law, F.C.P. and Farquharson, T. 1980. Phencyclidine and piperidinocyclohexane carbonitrile metabolism by the isolated perfused lung and lung microsomes of rabbits. Fed. Proc. 39: 756.

Law, F.C.P. and So, E.M.K. 1981. Induction studies of Δ^1-tetrahydrocannabinol metabolism by rat and rabbit lungs. Comp. Biochem. Physiol. 69C: 19-23.

Law, F.C.P., Eling, T.E., Bend, J.R. and Fouts, J.R. 1974. Metabolism of xenobiotics by the isolated perfused lung. Comparison with *in vitro* incubation. Drug. Metab. Dispos. 3: 433-442.

Lech, J.J. and Bend, J.R. 1980. Relationship between biotransformation and the toxicity and fate of xenobiotic chemicals in fish. Environ. Health Perspect. 34: 115-131.

Lee, I.P., Suzuki, K., Lee, S.D. and Dixon, R.L. 1980. Aryl hydrocarbon hydroxylase induction in rat lung, liver and male reproductive organ following inhalation exposure to diesel emission. Toxicol. Appl. Pharmacol. 52: 181-184.

Lidman, U., Förlin, L., Molander, O. and Axelson, G. 1976. Induction of the drug metabolizing system in rainbow trout (*Salmo gairdneri*) liver by polychlorinated biphenyls (PCBs). Acta Pharmacol. Toxicol. 39: 262-272.

Liem, H., Muller-Eberhard, U. and Johnson, E.F. 1980. Induction of cytochrome P-450 form 6 in rabbit kidney and lung by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Fed. Proc. 39: 1014.

Litterst, C.L., Mimnaugh, E.G. and Gram, T.E. 1977. Comparative alterations in extrahepatic drug metabolism by factors known to affect hepatic activity. Biochem. Pharmacol. 26: 749-755.

Lubawy, W.C. and Isaac, R.S. 1980. Influence of pretreatment with tobacco smoke condensate or 3-methylcholanthrene on [^{14}]benzo-(a)pyrene metabolism in the isolated perfused rabbit lung. Toxicol. Lett. 7: 153-159.

Lubawy, W.C. and Perrier, D.G. 1980. The effect of acute tobacco smoke exposure on pulmonary benzo[a]pyrene metabolism. Environ. Res. 21: 438-445.

Marcotte, J. and Witschi, H.P. 1972. Induction of pulmonary aryl hydrocarbon hydroxylase by marijuana. Res. Commun. Chem. Pathol. Pharmacol. 4: 561-568.

Matsubara, T., Prough, R.A., Burke, M.D. and Estabrook, R.W. 1974. The preparation of microsomal fractions of rodent respiratory tract and their characterization. Cancer Res. 34: 2196-2203.

Matsubara, T., Nakamura, Y. and Tochino, Y. 1975. Electron transport systems of lung microsomes and their physiological functions, enzymic hydroxylation of aniline and steroids. Xenobiotica 5: 205-212.

Mitchell, C.E. 1980. Induction of aryl hydrocarbon hydroxylase in Chinese hamsters and mice following intratracheal instillation of benzo(a)pyrene. Res. Commun. Chem. Pathol. Pharmacol. 28: 65-78.

Nakazawa, K. and Costa, E. 1971. The pharmacological implications of Δ^9-tetrahydrocannabinol metabolism by lung: effects of 3-methylcholanthrene. Ann. N.Y. Acad. Sci. 191: 216-221.

Okamoto, T., Chan, P.C. and So, B.T. 1972. Effects of tobacco, marijuana, and benzo[a]pyrene on aryl hydrocarbon hydroxylase in hamster lung. Life Sci. 11: 733-741.

Oppelt, W.W., Zange, M., Ross, W.E. and Remmer, H. 1970. Comparison of microsomal drug hydroxylation in lung and liver of various species. Res. Commun. Chem. Pathol. Pharmacol. 1: 43-56.

Payne, J.F. and May, N. 1979. Further studies on the effect of petroleum hydrocarbons on mixed-function oxidases in marine organisms. In Pesticide and xenobiotic metabolism in aquatic organisms. Edited by M.A.Q. Khan, J.J. Lech and J.J. Menn. American Chemical Society, Washington, D.C. pp. 339-347.

Payne, J.F. and Penrose, W.R. 1975. Induction of aryl hydrocarbon (benzo[a]pyrene) hydroxylase in fish by petroleum. Bull. Environ. Contamin. Toxicol. 14: 112-116.

Pedersen, M.G., Hershberger, W.K. and Juchau, M.R. 1974. Metabolism of 3,4-benzpyrene in rainbow trout (Salmo gairdneri). Bull. Environ. Contamin. Toxicol. 12: 481-486.

Pohl, R.J., Bend, J.R., Guarino, A.M. and Fouts, J.R. 1974. Hepatic microsomal mixed-function oxidase activity of several marine species from coastal Maine. Drug Metab. Dispos. 2: 545-555.

Pohl, R.J., James, M.O., Fouts, J.R. and Bend, J.R. 1976. Xenobiotic metabolism in marine fish treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 3-methylcholanthrene (3-MC), or 1,2,3,4-dibenzanthracene (DBA). Pharmacologist 18: 207.

Poore, R.E. and Neal, R.A. 1972. Evidence for extrahepatic metabolism of parathion. Toxicol. Appl. Pharmacol. 23: 759-768.

Pritchard, J.B., Guarino, A.M. and Kinter, W.B. 1973. Distribution, metabolism, and excretion of DDT and mirex by a marine teleost, the winter flounder. Environ. Health Perspect. 4: 45-54.

Reid, W.D., Ilett, K.F., Glick, J.M. and Krishna, G. 1973. Metabolism and binding of aromatic hydrocarbons in the lung. Am. Rev. Respir. Dis. 107: 539-551.

Remmer, H. 1975. Pulmonary drug-metabolizing enzymes. In Lung metabolism. Edited by A.F. Junod and R. deHaller. Academic Press, New York. pp. 133-158.

Ryan, A.J., James, M.O., Ben-Zvi, Z., Law, F.C.P. and Bend, J.R. 1976. Hepatic and extrahepatic metabolism of ^{14}C-styrene oxide. Environ. Health Perspect. 17: 135-144.

Sabo, D.J. and Stegeman, J.J. 1977. Some metabolic effects of petroleum hydrocarbons in marine fish. In Physiological responses of marine biota to pollutants. Edited by F.J. Vernberg, A. Calabrese, F.P. Thurberg and W.B. Vernberg. Academic Press, New York. pp. 279-287.

Scarpelli, D.G. 1977. General discussion to Part IV. Biological effects on marine animals: Health implication for man. Ann. N.Y. Acad. Sci. 298: 463-481.

Serabjit-Singh, C.J., Wolf, C.R. and Philpot, R.M. 1979. The rabbit pulmonary monooxygenase system: immunological and biochemical characterization of enzyme components. J. Biol. Chem. 254: 9901-9907.

Sieber, S.M. and Adamson, R.H. 1977. The metabolism of xenobiotics by fish. In Drug metabolism from microbe to man. Edited by D.V. Park and R.L. Smith. Taylor and Francis Ltd., London. pp. 233-245.

Smith, B.R., Philpot, R.M. and Bend, J.R. 1978. Metabolism of benzo[a]pyrene by the isolated perfused rabbit lung. Drug Metab. Dispos., 6: 425-431.

Sorokin, S.P. 1970. The cells of the lungs. In Morphology of experimental respiratory carcinogenesis. Edited by P. Nettesheim, M.G. Hanna Jr. and J.W. Deatherage Jr. U.S. Atomic Energy Commission, Division of Technical Information, Oak Ridge, Tenn. pp. 3-43.

Statham, C.N., Elcombe, C.R., Szyjka, S.P. and Lech, J.J. 1978. Effect of polycyclic aromatic hydrocarbons on hepatic microsomal enzymes and disposition of methylnaphthalene in rainbow trout in vivo. Xenobiotica 8: 65-71.

Stegeman, J.J. 1978. Influence of environmental contamination on cytochrome P-450 mixed-function oxygenases in fish: implications for recovery in the Wild Harbor Marsh. J. Fish. Res. Board Can. 35: 668-672.

Sunderman, F.W. Jr. 1967. Inhibition of induction of benzpyrene hydroxylase by nickel carbonyl. Cancer Res. 27: 950-955.

Tucker, L.D. II and Shertzer, H.G. 1980. Split lung perfusion: a new method to test the effects of chemical agent on pulmonary metabolism. Toxicol. Appl. Pharmacol. 55: 353-358.

Uehleke, H. 1969. Extrahepatic microsomal drug metabolism. In Proceedings of the European Society for the Study of Drug Toxicity, Vol. 10--Sensitization to drugs. Edited by S.B. Baker and J. Tripod. Excerpta Medica Foundation, Amsterdam. pp. 94-100.

Uehleke, H. 1971. N-hydroxylation. Xenobiotica 1: 327-338.

Uotila, P., Hartiala, J. and Aitio, A. 1977. Cigarette smoke: inducer of glucuronide synthesis in the isolated perfused rat lung. Toxicol. Appl. Pharmacol. 41: 445-448.

Vähäkangas, K., Nevasaari, K., Pelkonen, O. and Kärki, N.T. 1979. Effects of various in vitro-inhibitors of benzo[a]pyrene metabolism in isolated rat lung perfusion. Acta Pharmacol. Toxicol. 45: 1-8.

Van Cantfort, J. and Gielen, J. 1975. Organ specificity of aryl hydrocarbon hydroxylase induction by cigarette smoke in rats and mice. Biochem. Pharmacol. 24: 1253-1256.

Van Cantfort, J. and Gielen, J. 1977. Induction by cigarette smoke of aryl hydrocarbon hydroxylase activity in the rat kidney and lung. Int. J. Cancer 19: 538-545.

Warshawsky, D., Niemeier, R.W. and Bingham, E. 1978. Influence of particulates on metabolism of benzo[a]pyrene in the isolated perfused lung. In Polynuclear aromatic hydrocarbons: Second International Symposium on analysis, chemistry and biology. Edited by P.W. Jones and R.I. Freundenthal. Raven Press, New York. pp. 347-360.

Wattenberg, L.W., Leong, J.L. and Galbraith, A.R. 1968a. Induction of increased benzpyrene hydroxylase activity in pulmonary tissue in vitro. Proc. Soc. Exp. Biol. Med. 127: 467-469.

Wattenberg, L.W., Page, M.A. and Leong, J.L. 1968b. Induction of increased benzpyrene hydroxylase activity by flavones and related compounds. Cancer Res. 28: 934-937.

Wattenberg, L.W., Page, M.A. and Leong, J.L. 1968c. Induction of increased benzpyrene hydroxylase activity by 2-phenylbenzothiazoles and related compounds. Cancer Res. 28: 2539-2544.

Welch, R.M., Loh, A. and Conney, A.H. 1971. Cigarette smoke: stimulatory effect on the metabolism of 3,4-benzpyrene by enzymes in rat lung. Life Sci. 10: 215-221.

Welch, R.M., Cavallito, J. and Loh, A. 1972. Effect of exposure to cigarette smoke on the metabolism of benzo[a]pyrene and acetophenetidin by lung and intestine of rats. Toxicol. Appl. Pharmacol. 23: 749-758.

Whitnack, E., Knapp, D.R., Holmes, J.C., Fowler, N.O. and Gaffney, T.E., 1972. Demethylation of nortriptyline by the dog lung. J. Pharmacol. Exp. Ther. 181: 288-291.

Wiebel, F.J., Leutz, J.C., Diamond, L. and Gelboin, H.V. 1971. Aryl hydrocarbon (benzo[a]pyrene hydroxylase) in microsomes from rat tissues: differential inhibition and stimulation by benzoflavones and organic solvents. Arch. Biochem. Biophys. 144: 78-86.

Witschi, H. and Saint-Francois, B. 1972. Enhanced activity of benzpyrene hydroxylase in rat liver and lung after acute cannabis administration. Toxicol. Appl. Pharmacol. 23: 165-168.

Wolf, C.R., Szutowski, M.M., Ball, L.M. and Philpot, R.M. 1978. The rabbit pulmonary monooxygenase system: characteristics and activities of two forms of pulmonary cytochrome P-450. Chem.-Biol. Interact. 21: 29-43.

Yarbrough, J.D. and Chambers, J.E. 1977. Crude oil effects on microsomal mixed-function oxidase system components in the striped mullet (Mugil cephalus). Life Sci. 21: 1095-1100.

Zitting, A. 1980. The effects of the fumes from heated rosin (Colophony) on tissue glutathione and xenobiotic metabolism in rat lung and liver. Res. Commun. Chem. Pathol. Pharmacol. 27: 563-569.

THE EFFECT OF ASBESTOS ON THE BIOACTIVATION OF POLYNUCLEAR AROMATIC HYDROCARBONS

C. Kandaswami[a] and P.J. O'Brien[a]

ABSTRACT

Asbestos, a naturally occurring mineral polymer which is almost ubiquitously distributed in the present-day environment, appears to exacerbate the effects of polynuclear aromatic hydrocarbons. The cellular and molecular bases of this synergistic effect remain to be elucidated. The studies in our laboratory indicate that mineral particulates enhance the uptake of benzo(a)pyrene and impair liver and lung microsomal aryl hydrocarbon hydroxylases in vitro. This effect might have important consequences in vivo. By retarding the metabolic disposition of benzo(a)pyrene, the particles could increase the residence time of the hydrocarbon in the lung, and consequently accentuate the carcinogenic stimulus. Examination of the benzo(a)pyrene metabolites generated by rat lung microsomes, in the presence of particulates, reveals a reduction in phenolic metabolites, dihydrodiols, and more polar compounds. Thus, the particulates appear to modify aryl hydrocarbon hydroxylase and epoxide metabolizing enzymes. Similar results were obtained with mouse embryo fibroblast cells. When mouse embyro fibroblasts are treated with UICC Canadian chrysotile for 24 hours and then grown in the presence of benzo(a)pyrene, there is a depression in the metabolism of the hydrocarbon in the cells. While decreasing the metabolism of benzo(a)pyrene, the particles appear to augment the binding of benzo(a)pyrene metabolites to cellular macromolecules. Preliminary studies indicate that Canadian chrysotile exerts a more pronounced effect than crocidolite on the cytotoxicity of benzo(a)pyrene in cell culture.

[a] Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland.

EFFET DE L'AMIANTE SUR L'ACTIVATION BIOLOGIQUE DES HYDROCARBURES AROMATIQUES POLYCYCLIQUES

C. Kandaswami[a] et P.J. O'Brien[a]

RESUME

L'amiante, un polymère minéral naturel que l'on trouve pratiquement partout dans l'environnement actuel, semble accroître les effets des hydrocarbures aromatiques polycycliques. La cause moléculaire de cet effet synergique n'a pas encore été trouvée. Les études effectuées dans notre laboratoire montrent que les particules minérales favorisent l'absorption du benzo(a)pyrène et réduisent, in vitro, l'activité des aryl-hydrocarbures-hydroxylases des microsomes du foie et du poumon. Cet effet pourrait avoir des conséquences importantes in vivo. Les particules, en retardant l'élimination métabolique du benzo(a)pyrène, pourraient accroître le temps de séjour de l'hydrocarbure dans le poumon et donc accentuer le stimulus carcinogène. L'étude des métabolites du benzo(a)pyrène produits par les microsomes du poumon chez le rat en présence de particules indique une réduction des métabolites phénoliques, des dihydrodiols et de composés plus polaires. Les particules semblent donc modifier l'aryl-hydrocarbure-hydroxylase et les enzymes métabolisant les époxydes. On a obtenu de résultats semblables avec des fibroblastes d'embryons de souris. On note une diminution du métabolisme de l'hydrocarbure quand des fibroblastes d'embryons de souris sont traités pendant 24 heures par de la chrysotile canadienne de l'UICC l'être cultivés en présence de benzo(a)pyrène. Les particules semblent accroître la fixation des métabolites du benzo(a)pyrène aux macromolécules cellulaires tout en diminuant le métabolisme de cette substance. D'après des études préliminaires, la chrysotile canadienne a un effet plus prononcé que las crocidolite sur la cytotoxicité du benzo(a)pyrène dans la culture de cellules.

[a] Département de biochimie, Université Memorial de Terre-Neuve, St. John's (Terre-Neuve).

INTRODUCTION

Polynuclear aromatic hydrocarbons represent one of the most widespread groups of all known chemical environmental contaminants (Weisburger and Williams 1980). Many of these pollutants are recognized to be carcinogenic or cocarcinogenic, apparently as a result of oxidative biotransformations in the body (Dipple 1976). The increasing incidence of respiratory cancers has been attributed to the bioactivation of polynuclear aromatic hydrocarbons prevalent in the ecosystem (Hammond et al. 1976). Some of these hydrocarbons present in cigarette smoke, e.g. benzo(a)pyrene (BP) and benz(a)anthracene, appear to be potent carcinogens in experimental animals (Harris 1977; Severson et al. 1978). Even though polycyclic aromatic hydrocarbons have been incriminated in the genesis of lung cancer, this intricate process seems to involve the interplay of multiple environmental factors (Epstein 1974; Harris 1977; Laskin et al. 1970). Incidentally, these hydrocarbons are not particularly effective in inducing bronchogenic carcinoma in animals unless administered in the presence of particulates or irritants (Laskin et al. 1970; Saffiotti et al. 1968).

The action of polycyclic aromatic hydrocarbons introduced into the bronchial tree could be considerably amplified by the simultaneous presence of asbestos fibers (Shabad et al. 1974). This action would explain the synergistic effect of asbestos exposure and cigarette smoking, as exemplified by an increased incidence of bronchogenic carcinoma in asbestos-exposed individuals who smoke cigarettes (Selikoff and Lee 1978). Asbestos, a group of naturally occurring magnesium silicate fibrous substances, is ubiquitously distributed in the contemporary environment (Selikoff and Lee 1978; Shugar 1979), and is potentially hazardous to man (Harington et al. 1975; Harington 1976; Rahman et al. 1977; Saffiotti and Wagoner 1976; Selikoff and Lee 1978). Information is scarce on the mechanism by which asbestos fibers and polynuclear aromatic hydrocarbons interact to cause the synergistic effect which modifies the overt toxicity of these hydrocarbons.

Several mechanisms could be invoked to account for particle-polycyclic aromatic hydrocarbon carcinogenesis. Pylev and co-workers (1969) and Shabad et al. (1974) demonstrated that asbestos could readily adsorb BP in vitro and decrease its excretion in experimental animals. By adsorbing the carcinogen onto its surface, the mineral particle may function as a carrier for the transport of the compound into the pulmonary system in a state that cannot be readily cleared. If asbestos were to retard the metabolic disposition of BP, this action would increase the effective dose of the carcinogen and prolong the duration of its contact with the site of metabolic activation.

The metabolism of BP and its congeners is mediated by aryl hydrocarbon hydroxylase (AHH), a cytochrome P-450-linked mixed function oxidase system induced by a multitude of xenobiotic stimuli (Nebert 1980). High levels of this enzyme result in rapid metabolism of carcinogenic hydrocarbons to excretable products (Benedict et al. 1972;

Kouri et al. 1973). However, some of the reactive intermediates generated transiently during this process could be more carcinogenic than the parent compound itself. Thus, the AHH system is associated with the detoxification or activation of polynuclear aromatic hydrocarbons in chemical carcinogenesis.

The degree and duration of the xenobiotic effect may be, therefore, dependent on a delicate balance between toxication and detoxification at the site of action. By modifying AHH activity, mineral fibers might alter the toxic potential of the carcinogen. Experiments underway in our laboratory show that asbestos fibers could modify AHH activity (Kandaswami and O'Brien 1980, 1981). The question arises whether such an effect of the mineral is due to action of the particle per se or is caused by contaminant heavy metals in the fibers. Trace metals, present in asbestos as natural impurities or added during processing, may be released in biologically active forms when the fibers are deposited in soft tissues (Cralley et al. 1967, 1968; Cralley and Lainhart 1973; Morgan and Cralley 1974). Dixon et al. (1970) proposed that some of these heavy metals could inhibit BP hydroxylase and thus increase the carcinogenic stimulus of the hydrocarbon. Such an action could amplify the synergistic effect of asbestos and hydrocarbons in the production of pulmonary carcinoma. It would be imperative, therefore, to investigate whether asbestos-associated metals would contribute to or alter this synergistic effect. In the present paper we address some of the aforementioned issues with a view to gaining an insight into factors which could modify the bioactivation of polynuclear aromatic hydrocarbons.

STUDIES WITH MICROSOMAL PREPARATIONS

EFFECT OF ASBESTOS ON ARYL HYDROCARBON HYDROXYLASE

In an effort to study the effect of particulates on the cellular transport and metabolic activation of polycyclic aromatic hydrocarbons, we examined the effect of asbestos on BP uptake by microsomal membranes and lipid micelles. Asbestos, when coated with BP, mediated a rapid transport of the carcinogen into the membrane. The consequence of the enhanced uptake of the hydrocarbon was a modification of BP hydroxylation (Kandaswami and O'Brien 1980).

Asbestos causes a marked inhibition of AHH activity of liver microsomes. Canadian chrysotile B and crocidolite appeared to be very effective inhibitors of this activity in rabbit and rat liver microsomes as illustrated in Table 1. Maximal enzyme inhibition was observed with Canadian chrysotile in all the microsomal fractions examined. In these experiments BP was pretreated with asbestos. Treatment of rat lung microsomal fraction with asbestos resulted in a marked diminution of AHH activity (Table 2). Crocidolite appears to cause the most inhibition of the lung microsomal hydroxylase. Adsorption of BP onto asbestos also

Table 1. Effect of asbestos on aryl hydrocarbon hydroxylase activity in liver microsomes.[α]

Addition	Specific Activity 3-Hydroxybenzo(a)pyrene formed (ρmoles/min/mg of protein)		
	Rabbit	Rat[β]	Rat(3-MC)[γ]
None	36.0	90.0	500.0
Crocidolite	15.9	44.1	380.0
Canadian chrysotile B	11.2	40.5	260.0
Amosite	22.0	55.8	420.0
Anthophyllite	22.9	63.9	315.0

[α] Aryl hydrocarbon hydroxylase was assayed fluorometrically. Union International Centre le Cancer (UICC) standard reference samples of asbestos were used. Benzo(a)pyrene was pretreated with asbestos (25 µg/mL). The experimental conditions were as described by Kandaswami and O'Brien (1980). The values represent averages of three separate experiments.

[β] Control rat liver microsomes were used.

[γ] 3-Methylcholanthrene-treated rat liver microsomes were used.

Table 2. Effect of asbestos on aryl hydrocarbon hydroxylase activity of 3-methylcholanthrene-pretreated rat lung microsomes.[α]

Addition	Specific Activity 3-Hydroxybenzo(a)pyrene formed (ρmoles/min/mg of protein)
None	15.0
Crocidolite	5.4
Canadian chrysotile B	6.3
Amosite	6.2
Anthophyllite	6.5

[α] The experimental conditions were as described by Kandaswami and O'Brien (1981). Asbestos (1.0 mg) was pre-incubated with the microsomal fraction for 1 hour. The values represent averages of three separate experiments.

depressed AHH activity of lung microsomes (Table 3). However, low doses of asbestos were found to inhibit the activity.

In the aforementioned studies, the assay for AHH was performed in the presence of the metal chelating agent, EDTA. As shown, in Table 4, AHH activity of rat liver microsomes is greatly diminished by Cu^{2+} in the absence of EDTA. However, if the microsomal incubation is performed in the presence of EDTA, the inhibitory effect of Cu^{2+} is abolished. When liver microsomes are treated with asbestos in the absence of EDTA, the inhibition of AHH is less pronounced. If asbestos-associated metals were to impair this activity, one would expect a greater inhibition of the activity in the absence of the metal chelating agent. Asbestos is effective in modifying AHH activity at lower concentrations as illustrated in Table 5. With increasing concentrations of the fiber, there was a progressive decline in inhibition of AHH activity.

MODIFICATION OF MICROSOMAL ARYL HYDROCARBON HYDROXYLASE ACTIVITY BY METAL IONS

Heavy metals have been reported to inhibit the activity of microsomal AHH (Aitio et al. 1978; Thomson et al. 1974; Tsang and Furst 1976). It was of interest to compare the effect of metal ions on this activity with that elicited in response to particulates. Cr^{3+}, Fe^{2+}, and Co^{2+} at a concentration of 10 μM markedly inhibited AHH activity of 3-methylcholanthrene-pretreated rat liver microsomes (Table 6). Ni^{2+} and Mn^{2+} did not impair this activity significantly at this concentration. At higher concentrations, both these metal ions caused some stimulation of the hydroxylase. Such an effect is different from that observed in the presence of different concentrations of asbestos (Table 5). Tsang and Furst (1976) noted a slight stimulation of mouse liver AHH activity by Fe^{2+} at concentrations above 0.1 mM.

Heavy metals such as Ni^{2+}, Cr^{3+}, and Cd^{2+}, known to contaminate asbestos samples (Roy-Chowdhury et al. 1973), inhibit AHH activity of 3-methylcholanthrene-pretreated rat lung microsomes at a concentration of 10 μM. Of these trace metals, Ni^{2+} and Cd^{2+} are carcinogenic to rodents (Costa 1980; Sunderman 1978; Tsang and Furst 1976). Cd^{2+} is a more potent carcinogen than Ni^{2+}.

The effect of Ni^{2+} and Cd^{2+} on AHH activity of lung microsomes of 3-methylcholanthrene pretreated rats are depicted in Table 7. $NiSO_4$, at a concentration of 10^{-8} M, causes 39% inhibition of the enzyme activity. There is no increase in the degree of enzyme inhibition at higher Ni^{2+} concentrations. Thus, Ni^{2+} causes only a limited inhibition of the hydroxylase. At a Ni^{2+} concentration of 10^{-6} M, the inhibition is less pronounced. However, a second inhibition of the hydroxylase is found above this concentration. Dixon et al. (1970) also noticed inhibition of AHH activity in lung homogenates of untreated rats at both low and high Ni^{2+} concentrations.

Table 3. Effect of pretreatment of benzo(a)pyrene with asbestos on aryl hydrocarbon hydroxylase activity of 3-methylcholanthrene-pretreated rat lung microsomes.[α]

Addition	Specific Activity 3-Hydroxybenzo(a)pyrene formed (pmoles/min/mg of protein)
Control	15.0
Crocidolite	8.7
Canadian chrysotile B	9.6

[α] Benzo(a)pyrene was pretreated with asbestos as described by Kandaswami and O'Brien (1981). (The final concentration of asbestos was 25 μg/mL). The values represent averages of three separate experiments.

Table 4. Effect of EDTA on aryl hydrocarbon hydroxylase activity of rat liver microsomes.[α]

Addition	Specific Activity 3-Hydroxybenzo(a)pyrene formed (pmoles/min/mg of protein)
None	90.0
EDTA (0.2 mM)	94.5
Canadian chrysotile B	53.1
Canadian chrysotile B and EDTA (0.2 mM)	41.5
Cu^{2+} (10 μM)	26.1
Cu^{2+} (10 μM) and EDTA (0.2 mM)	87.7

[α] Benzo(a)pyrene was pretreated with asbestos. Experimental conditions were as described previously (Kandaswami and O'Brien 1980). The values represent averages of two separate experiments.

Table 5. Effect of asbestos concentration on aryl hydrocarbon hydroxylase activity of rat liver microsomes.[α]

Canadian Crysotile B Concentration (μg/mL)	Specific Activity 3-Hydroxybenzo(a)pyrene formed (pmoles/min/mg of protein)
0	90.0
25	32.4
50	43.2
75	62.1
100	68.4
125	81.0

[α] Benzo(a)pyrene was pretreated with the indicated amount of asbestos (Canadian chrysotile B) sample. Other experimental conditions were as described by Kandaswami and O'Brien (1980). The values represent averages of two separate experiments.

Table 6. Effect of 10 μM metal ions on aryl hydrocarbon hydroxylase activity of 3-methylcholanthrene-pretreated rat liver microsomes.[α]

Addition	Specific Activity 3-Hydroxybenzo(a)pyrene formed (pmoles/min/mg of protein)
None	500.0
$MnSO_4$	473.5
$NiSO_4$	467.0
$Cr_2(SO_4)_3$	160.0
$FeSO_4$	212.5
$CdCl_2$	200.0

[α] The values represent averages of three separate experiments.

Table 7. Effect of nickel and cadmium salts on aryl hydrocarbon hydroxylase activity of 3-methylcholanthrene-pretreated rat lung microsomes.[α]

Metal Ion Concentration	Relative Activity (%)[β]			
	$NiSO_4$	$Cd(CH_3COO)_2$	$CdSO_4$	$CdCl_2$
10^{-8} M	61.6	42.2	71.2	31.8
10^{-7} M	68.4	56.3	68.1	30.3
10^{-6} M	84.9	64.7	63.6	33.3
10^{-5} M	64.7	43.6	72.6	24.2
10^{-4} M	63.0	57.7	62.1	31.8
10^{-3} M	61.1	61.6	42.6	16.6

[α] The experimental conditions were as described previously (Kandaswami and O'Brien 1981).

[β] Expressed as a percentage of the aryl hydrocarbon hydroxylase activity of the control, containing no metal. The values represent averages of three separate experiments.

Cd^{2+} is found to be more inhibitory to lung microsomal AHH than is Ni^{2+}. Cadmium acetate, at a concentration of 10^{-8}M, depresses the enzyme activity by 58%. As the concentration of this ion is increased there is less inhibition. However, at 10^{-5} M, the degree of enzyme inhibition obtained is similar to that at 10^{-8} M. There is reduction in the inhibition of the hydroxylase at concentrations above 10^{-5} M. $CdSO_4$ is less inhibitory to the hydroxylase than is cadmium acetate. $CdSO_4$ at a concentration of 10^{-6} M causes 36% enzyme inhibition. Considerable inhibition is observed only at a concentration as high as 10^{-3} M. Of the different cadmium salts examined, $CdCl_2$ caused the maximal depression of AHH activity. At a concentration of 10^{-8} M, $CdCl_2$ diminished this activity by 68%. Higher concentrations of this salt produced a similar diminution of enzyme activity. However, 1 mM $CdCl_2$ reduced the hydroxylase activity by 83%. In contrast to the present observation, Aitio et al. (1978) reported that of the different cadmium salts examined, only CdI_2 inhibited this activity in untreated rat liver microsomes in vitro.

EFFECT OF PARTICULATES ON THE MICROSOMAL GENERATION OF BENZO(a)PYRENE METABOLITES

Several studies have shown that BP is oxidatively transformed to a complex array of metabolites which include arene oxides, phenols, quinones, and dihydrodiols (Holder et al. 1974; Selkirk et al. 1974; Sims and Grover 1974). The frequently used fluorescence technique for the determination of AHH yields no information on the production of dihydrodiols and other more polar compounds. The metabolites of ^{3}(H)-BP produced in the in vitro microsomal incubation were separated by thin layer chromatography and analyzed by standard methods (Grover et al. 1974; Sims 1970). The effect of asbestos on the formation of BP metabolites is summarized in Table 8. In this table, the major BP metabolites are classified into group-B (phenolic compounds and quinones) and group-C (dihydrodiols and other more polar compounds) metabolites. The reduction in the formation of metabolites caused by different types of asbestos is striking. These fibers decreased the production of both group-B and group-C metabolites. The phenolic compounds (belonging to group-B) are formed from the epoxides by a non-enzymic process while the dihydrodiols are generated enzymically. The increase in the B/C ratio indicates that asbestos inhibits BP-epoxide-metabolizing enzymes besides AHH.

EFFECT OF METAL IONS ON THE MICROSOMAL PRODUCTION OF BENZO(a)PYRENE METABOLITES

The common trace metals associated with asbestos fibers include Ni, Cr, Mn, Co, Fe, and Cd. An understanding of the modifying effect of these metals on the bioactivation of BP is essential. The percentages of BP metabolites formed with 3-methylcholanthrene-

Table 8. Effect of mineral particles on the formation of benzo(a)pyrene metabolites by 3-methylcholanthrene-pretreated rat lung microsomes.[α]

Condition	Percentage of the total amount of extracted radioactivity		
	Group B metabolites	Group C metabolites	Ratio of B/C
Control	17.1	14.9	1.15
Canadian chrysotile	2.0	1.4	1.43
Amosite	4.0	1.5	2.67
Anthophyllite	2.0	1.6	1.25

[α] The asbestos sample (1.0 mg) was pretreated with microsomes for 1 hour. Other experimental conditions were as described by Kandaswami and O'Brien (1981). Microsomes were incubated with 20 μM (^{3}H) benzo(a)pyrene for 15 minutes at 37°C. The products were extracted with acetone: ethyl acetate (1:2, v/v) and the metabolites were separated by thin layer chromatography on silica gel G plates. The following groups of compounds were separated by this procedure: benzo(a)pyrene, phenolic compounds and quinones (group B), dihydrodiols and other more polar compounds. (group C).

pretreated rat liver microsomes in the presence of various metal ions are shown in Table 9.

The rate of BP metabolism was markedly affected by Ni^{2+}, Cr^{3+}, and Fe^{3+}. Ni^{2+} and Cr^{3+} decreased the relative proportions of both group-B and group-C metabolites. Other metals examined, such as Mn^{2+}, Cd^{2+}, Fe^{2+}, and Co^{2+}, diminished the metabolism of BP to a lesser degree. Of these metal ions, only Mn^{2+} was effective in reducing the group-C metabolites. Therefore, Cd^{2+}, Fe^{2+} and Co^{2+} do not seem to impair BP-epoxide-metabolizing activities. Canadian chrysotile decreased the formation of both groups of BP metabolites. The effect of both metal ions and asbestos upon the metabolism of BP was also examined. The overall metabolism of BP in the presence of chrysotile was reduced by 10-15% by the treatment of the fibers with Cr^{3+} or Fe^{3+}. In this case, the inhibitory effect was more on the formation of group-B metabolites. The effect of all these metal ions on the production of BP metabolites in rat lung microsomes was also examined. Of the metals tested, Ni^{2+}, Cr^{3+}, Fe^{2+} and Fe^{3+} depressed the metabolism of BP significantly and, except in the case of Fe^{2+}, the effect was similar to that noted in liver microsomes. The addition of Ni^{2+}, Cr^{3+}, Fe^{2+}, or Fe^{3+} to chrysotile resulted in a slight decrease in the production of BP metabolites. $CdCl_2$ was not as effective as $NiSO_4$ in impairing the production of BP metabolites while the former was far more inhibitory in the fluorescence assay for AHH.

EFFECT OF ASBESTOS AND TRACE METALS ON THE MACROMOLECULAR BINDING OF BENZO(a)PYRENE METABOLITES

Canadian chrysotile caused a significant stimulation (about 40%) of the *in vitro* binding of microsomally-generated (^3H)-BP metabolites to the macromolecular fraction. Of the different metals tested, only Cr^{3+} and Mn^{2+} showed a similar effect. In this experiment the covalent binding of the metabolites to the macromolecular fraction of 3-methylcholanthrene-pretreated rat liver microsomes was performed as described by Hill and Shih (1975).

STUDIES WITH CULTURED CELLS

To further investigate the effect of asbestos fibers on the metabolic activation of BP, we have extended our work to mouse embryo fibroblasts. We exposed the cell cultures to (^3H)-BP and Canadian chrysotile and examined the metabolites of BP formed and the total binding of the metabolites to the macromolecules in the cells.

CELLULAR METABOLISM OF BENZO(a)PYRENE IN FIBROBLASTS

In one experiment, growing cells were harvested and exposed to Canadian chrysotile for 2 hours, then incubated with (^3H)-BP for one

Table 9. Effect of 1 mg asbestos and 10 μM metal salts on the production of benzo(a)pyrene metabolites by rat liver microsomes.[α]

Addition	Percentage of the total amount of extracted radioactivity	
	Group B metabolites	Group C metabolites
None	62.0	17.0
Canadian chrysotile	31.0	9.9
$NiSO_4$	28.0	7.7
$Cr_2(SO_4)_3$	29.7	6.7
$MnCl_2$	35.8	8.6
$CdCl_2$	44.5	14.3
$FeSO_4$	38.6	21.0
$CoCl_2$	43.1	16.7
$FeCl_3$	26.4	9.0

[α] The experimental conditions were as described in Table 8.

hour, and the metabolites were isolated. Analysis of the organic soluble metabolites revealed a 30% drop in the phenolic metabolites (group-B), as shown in Table 10. Similar results were obtained with (^{14}C)-7,12-dimethylbenz(a)anthracene. In another experiment (Table 11), cells were grown in the presence of (^{3}H)-BP for 72 hours with and without the fibers. When the cells were treated with Canadian chrysotile for 24 hours before adding BP, there was a reduction in BP metabolism. The phenolic metabolites (group-B) and polar compounds (group-C) were reduced by 55% and 40% respectively in comparison with cells treated with BP alone (control). When the cells were exposed to the hydrocarbon and asbestos simultaneously and maintained for 72 hours, there was no change in the metabolism. However, the relative proportions of the metabolites seemed to be altered. The percentage of phenolic metabolites (group-B) was increased. This may be due to the inhibition of BP epoxide-metabolizing enzymes. The proportions of group-C metabolites was slightly decreased. Eneanya et al. (1979) noticed a decrease in the overall metabolism of BP in Syrian hamster embryo cells treated with NIEHS intermediate chrysotile. On the other hand, there was no change in the metabolism of the hydrocarbon in human foreskin fibroblasts when the cells were incubated with (^{3}H)-BP and NIEHS intermediate chrysotile for 72 hours (Hart et al. 1980).

MACROMOLECULAR BINDING OF BENZO(a)PYRENE METABOLITES IN FIBROBLASTS

Treatment of fibroblasts with (^{3}H)-BP and Canadian chrysotile for 72 hours showed a limited increase (about 20%) in the binding of BP metabolites to the macromolecular fraction of the cell, as compared with that obtained in cells treated with the hydrocarbon alone. However, if asbestos was added to the cells 24 hours before the hydrocarbon treatment, there was more than a twofold stimulation of the binding of BP metabolites to macromolecular components. These results may indicate the modification of BP bioactivation by asbestos fibers. Eneanya et al. (1979) noticed an augmented association of BP metabolites with DNA when Syrian hamster embryo cells were treated with (^{3}H)-BP and chrysotile intermediate asbestos for 24 hours.

CYTOTOXICITIES OF BENZO(a)PYRENE AND ASBESTOS

The cytotoxic effects of asbestos and BP were evaluated by determining the percentage of surviving mouse embryo fibroblasts cells. The cell cultures were treated with asbestos or BP, or both, for a certain period of time and thereafter, the cells were washed and incubated in growth medium. Colony formation was allowed to proceed for 7-10 days. The cells were then harvested and were counted to assess cytotoxicity. Preliminary studies indicated that crocidolite was more cytotoxic than Canadian chrysotile when the cells were exposed to asbestos fibers alone. However, in the presence of BP the cytotoxic effect of chrysotile appeared to be more pronounced than that of crocidolite. These results indicate that reactive groups on different

Table 10. Metabolites formed on incubation of mouse embryo fibroblasts with (^{3}H) benzo(a)pyrene.[α]

Conditions	Percentage of the total amount of extracted radioactivity	
	Group-B metabolites	Group-C metabolites
Control	14.36	5.33
Canadian chrysotile-treated[β]	10.12	4.50

[α] Mouse embryo fibroblasts (3T6) were grown in Dulbecco's modified Eagle's medium at 37°C with 5% CO_2 atmosphere for 2 days. Thereafter the cells were treated with 1.3 μM (^{3}H)-benzo(a)pyrene for 1 hour. Metabolites were isolated and separated as described in Table 8.

[β] The cells were treated with Canadian chrysotile (1.0 mg) for 2 hours prior to the addition of benzo(a)pyrene.

Table 11. Benzo(a)pyrene metabolites formed in mouse embryo fibroblasts in 72 hours.[α]

Conditions	Percentage of the total amount of extracted radioactivity	
	Group-B metabolites	Group-C metabolites
1. Cells exposed to benzo(a)pyrene for 72 hours	13.01	40.06
2. Cells pre-exposed to Canadian chrysotile for 24 hours and then exposed to benzo(a)pyrene for 72 hours	5.80	24.13
3. Cells exposed simultaneously to Canadian chrysotile and benzo(a) pyrene for 72 hours	20.46	32.14

[α] Mouse embryo fibroblasts cell cultures, grown in 30 mL of Dulbecco's modified Eagle's medium to 60-80% confluency, were treated with 1.3 μM (^{3}H)-benzo(a)pyrene in the presence of Canadian chrysotile (30 μg/mL). The cultures were harvested and the metabolites of benzo(a)pyrene were isolated and analyzed as described in Table 8.

forms of asbestos might have differential effects on the bioactivation of BP.

SYNERGISTIC EFFECT OF MINERAL PARTICLES AND POLYNUCLEAR AROMATIC HYDROCARBONS: POSSIBLE MECHANISMS

The metabolism of BP is extremely complex. It is converted by microsomal mixed function oxidases (BP hydroxylase or AHH) and epoxide hydratase(s) to a bewildering array of phenols, quinones, epoxides, and dihydrodiols (Holder et al. 1974; Selkirk et al. 1974; Sims and Grover 1974). Some of these metabolites may be further transformed by conjugation mechanisms or recycled by oxidative reactions. Thus, toxicity and detoxification may be dependent on a balance of reactions resulting in the generation of primary metabolites and their further transformation. For the metabolism of polycyclic aromatic hydrocarbons one could envisage, under normal conditions, the enzymatic conversion of the parent compound to the epoxide intermediates, which subsequently, can be converted into the noncarcinogenic hydroxy forms. The detoxification of these hydrocarbons in the body might proceed in this fashion. The modifying effect of asbestos on AHH acitivity could have important consequences in vivo. Interferance of asbestos with the detoxification of BP may result in a slower pulmonary clearance of the carcinogen, thereby increasing its retention time. This would explain the greater induction by polycyclic aromatic hydrocarbons of BP hydroxylase in lymphocytes isolated from asbestos workers than from controls, first reported by Naseem et al. (1978), and confirmed by us (Table 12).

Tishler et al. (1977) suggested that the high risk of lung cancer for asbestos workers who also smoke cigarettes, is due to carcinogenic hydrocarbons of cigarette smoke which concentrate on the fibers, enter the respiratory system, and consequently remain lodged in the lungs. With asbestos, large amounts of material are retained in the tissues, unlike other carcinogenic agents which are excreted or metabolized (Seidman et al. 1979). If the mineral were to retard the early metabolism of BP, the presence of retained asbestos may ensure a continuing exposure of the tissue to the carcinogen. Thus, BP may remain biologically active in the lung for a prolonged duration. The retention of unmetabolized BP in the lungs would accentuate the carcinogenic stimulus insofar as the risk of bronchogenic carcinoma correlates with the duration of exposure to the carcinogen (Shabad et al. 1974).

The observed modification of the metabolism of BP in fibroblasts by asbestos fibers further confirms our results obtained with the in vitro studies on the microsomal metabolism of BP. The metabolism of BP in the cells appears to be retarded by chrysotile when the fiber is added to the cell culture 24 hours before the addition of the hydrocarbon. This might be due to the retention of the carcinogen on the particle surface. The question arises whether fiber absorption

Table 12. Lymphoblast aryl hydrocarbon hydroxylase activity.[a]

Group	Basal activity	3-Methylcholanthrene-induced activity	Inducibility
Control Subjects (10)	0.19 ± 0.02	0.33 ± 0.1	1.7
Asbestos Miners (80)	0.17 ± 0.04	0.87 ± 0.3	5.1

[a] Activity values are expressed as pmoles of product/min/10^6. Results are given as mean ± S.D.

of the carcinogen is a prerequisite for efficient transport. Enhanced cellular uptake of carcinogen absorbed onto the particle surface has been considered as a partial explanation for cocarcinogenesis (Lakowicz and Bevan 1980). It would be instructive to investigate the effect of exposure of cell cultures to asbestos-adsorbed BP on the rate of BP metabolism.

Exposure of fibroblasts to Canadian chrysotile prior to hydrocarbon treatment appears to augment the macromolecular binding of BP metabolites in the cells, even though there is a decrease in the carcinogen metabolism. This might indicate that asbestos shifts metabolism to the formation of products possessing higher macromolecular binding potential. It could account for the synergistic effect since the carcinogenicity of BP has been ascribed to the binding of its metabolites to tissue macromolecules (Brookes 1975; Heidelberger 1973). Further work is needed to elucidate the effect of asbestos on BP metabolite binding to DNA and other cellular macromolecules in vivo.

It is now known that heavy metals, such as Ni, Cr, and Co, are found on the surface of asbestos in trace amounts (Roy-Chowdhury et al. 1973; Selikoff and Lee 1978). Dixon et al. (1970) have postulated that these metals can inhibit BP hydroxylase and indirectly contribute to a carcinogenic stimulus. Our preliminary studies, however, indicate that the in vitro effect of particles on AHH may not be due to associated heavy metals. Canadian chrysotile caused a reduction in the in vitro production of BP metabolites by rat liver microsomes. The pretreatment of chrysotile with heavy metals did not modify this effect to any large extent. Canadian chrysotile appears to increase the rat liver microsome-catalyzed irreversible binding of BP to macromolecules. Heavy metals were not particularly effective in this binding.

Our in vitro microsomal studies did not reveal any large differences in the effects of different types of fibers on the formation of BP metabolites as would be expected if fiber-associated metal ions were to contribute to this effect. Hart et al. (1980) observed an enhanced binding of BP metabolites to DNA in NIEHS intermediate chrysotile-pretreated human fibroblasts. Since this form of chrysotile undergoes least elemental change during intracellular uptake, the authors do not ascribe this modifying effects to metallic ions. In this study, leaching of Fe from crocidolite upon engulfment by fibroblasts was indicated. On this basis they predict that if elemental loss is involved in alteration of BP metabolism, then the synergistic effect of amosite and crocidolite would exceed that of chyrsotile. Stanton (1973) pointed out that there is a wide variation in the metallic content of asbestos samples that seem to bear no relation to their carcinogenicity. On the basis of available information, one cannot attribute the cocarcinogenic effect of asbestos fibers solely to fiber-associated heavy metals.

Trace metals and organic materials contaminating asbestos can be leached or absorbed from asbestos by biologic fluids (Jaurand et al.

1977). Substances present in the acid-extracted fraction of asbestos might be involved in cytotoxicity of the fiber, or could be responsible for blocking sites involved in toxicity depending on the form of asbestos (Reiss et al. 1980). Studies are proposed to examine the leaching of asbestos fibers in different media and the effect of both the post-leaching fluids and the leached fibers on the bioactivation and cytotoxicity of BP. It would be instructive to investigate the effects of such leached fibers on the induction of cancer by polycyclic aromatic hydrocarbons.

The role of toxic, carcinogenic, and other heavy metals in the modulation of AHH (Aitio et al. 1978; Tsang and Furst 1976) is of prime interest in the general context of combined effects of xenobiotics. Of the heavy metals studied for carcinogenic effects, Ni, Cr, and Cd appear to be potent carcinogens for rodents (Costa 1980; Furst 1977; Sunderman 1978). Ni and Cd have been reported to be present in cigarette smoke (Costa 1980). Individuals smoking cigarettes and also exposed to mineral particulates may be subjected to multiple effects. Our in vitro studies with rat liver microsomes indicate that Ni, Cr, and Cd impair AHH. Cr appears to inhibit epoxide-metabolizing enzymes also. It would be of interest to investigate the effect of pretreatment of experimental animals with asbestos as well as environmentally important heavy metals on the induction of AHH by polycyclic aromatic hydrocarbons.

Studies with cultured cells and in vitro experiments with microsomes indicate that asbestos could modify, in some way, the binding of BP metabolites to macromolecules. It would be instructive to study the effect of asbestos upon the in vivo covalent binding of BP to DNA.

Asbestos exposure appears to produce non-heritable chromosomal changes in cultured cells (Sincock 1976). Since the mutagenicity of asbestos has not been domonstrated, asbestos is not considered to function as a genotoxic carcinogen that induces chemical changes in DNA (Weisburger and Williams 1980). It needs to be investigated whether asbestos could promote the DNA-damaging action of chemical carcinogens. Hawco et al. (1980) have recently shown that hydroxyl radicals are formed during the metabolism of BP by microsomal mixed function oxidase. Nucleic acid strand breaks would be expected to occur if these hydroxyl radicals were generated near the nucleic acids (Hawco et al. 1980). Whether asbestos could enhance such hydroxyl radical formation remains to be uncovered.

SUMMARY AND CONCLUSIONS

Current evidence indicates that cigarette smoke is a major cause of bronchogenic carcinoma in man. The incidence of lung cancer has been attributed to the bioactivation of polynuclear aromatic hydrocarbons, for example BP and benz(a)anthracene. Several experimental studies show that the action of such carcinogenic hydrocarbons introduced to the bronchial tree is considerably

intensified by the simultaneous presence of asbestos fibers. These observations explain the synergistic effect of asbestos exposure and cigarette smoking. Pathological and epidemiological studies also show that asbestos is a cocarcinogen. However, the cellular and molecular bases of the interaction between polycyclic aromatic hydrocarbons and asbestos remain to be understood.

In the induction of pulmonary carcinoma, the retention of the carcinogen in the tissue would seem to be essential. Asbestos fibers may be effective in retarding the pulmonary clearance of carcinogenic hydrocarbons. It would be important to elucidate how the fibers could exert such an effect. The metabolism of polycyclic hydrocarbons, for example BP and benz(a)anthracene, is mediated by AHH. Factors which modify this activity may have a profound effect on the metabolic disposition of BP. Our studies indicate that asbestos modifies AHH activity of microsomes and whole cells. Our results indicate that asbestos could also alter the activities of epoxide metabolizing enzymes. These enzymes are involved in the metabolic disposition of polycyclic hydrocarbons. The above effects of asbestos may have important consequences *in vivo*. By retarding the rapid metabolism of the hydrocarbon, asbestos could increase the residence time of the carcinogen in the lung tissue.

Other possible explanations should also be considered for the interactions between asbestos and polycyclic aromatic hydrocarbons. It is likely that asbestos causes physical injury to the bronchial tree, thereby suppressing the mucociliary clearance. In such a suggestion, emphasis is placed on the role of asbestos as a physical irritant. However, nothing much is known about this action of inhaled fibers (Selikoff and Lee 1978). It is possible that asbestos may impair cellular immune response (Shugar 1979). The lack of immunological surveillance may render the body more susceptible to carcinogenic hydrocarbons. This possibility remains to be studied.

While decreasing the overall *in vitro* metabolism of BP, asbestos seems to enhance the binding of BP metabolites to macromolecules. It is likely that asbestos redirects the metabolism of BP along a different pathway. Further work concerning the effect of intratracheal instillation of asbestos in rats on the metabolism and binding of BP is in progress. In the presence of asbestos, polycyclic aromatic hydrocarbons could be increasingly activated by another enzyme system, such as the prostaglandin synthetase. Sirois *et al.* (1980) have shown that asbestos causes a high increase in alveolar macrophage phospholipase A activity and prostaglandin synthesis. Therefore, asbestos could act as a tumor promotor. Interestingly, the carcinogenic effect of asbestos has been attributed to its action as a promotor (Harington *et al.* 1975). Recently it has been indicated that asbestos acts like tumor promotors in inducing changes in the cyclic nucleotide concentrations in cell cultures (Tejwani *et al.* 1980). Further studies are contemplated on the effect of asbestos upon the bioactivation and binding of polynuclear aromatic hydrocarbons in alveolar macrophages.

ACKNOWLEDGMENTS

The financial support of the National Cancer Institute of Canada is gratefully acknowledged. We wish to thank Mrs. Linda Ross for her generous help in cell culturing, and Dr. R. Green for advice and for making his facilities available. We are thankful to Dr. Ronald W. Hart, Director, National Center for Toxicological Research, Jefferson, Arkansas, for kindly making his manuscripts available prior to publication. The assistance of Miss Loretta Brothers in the preparation of this manuscript is appreciated.

REFERENCES

Aitio, A., Ahotupa, M. and Parkki, M.G. 1978. Inhibition of drug metabolizing enzymes by heavy metals in vitro. Biochem. Biophys. Res. Commun. 83: 850-856.

Benedict, W.F., Gielen, J.E. and Nebert, D.W. 1972. Polycyclic hydrocarbon-produced toxicity, transformation and chromosomal aberrations as a function of aryl hydrocarbon hydroxylase activity in cell cultures. Int. J. Cancer 9: 435-451.

Brookes, P. 1975. Covalent interactions of carcinogens with DNA. Life Sci. 16: 331-344.

Costa, M. 1980. Metal carcinogenesis testing: principles and in vitro methods. The Humana Press Inc., Clifton, New Jersey. 164 pp.

Cralley, L.J. and Lainhart, W.S. 1973. Are trace metals associated with asbestos fibers responsible for the biological effects attributed to asbestos? J. Occup. Med. 15(3): 262-266.

Cralley, L.J., Keenan, R.G. and Lynch, J.R. 1967. Exposure to metals in the manufacture of asbestos textile products. Am. Ind. Hyg. Assoc. J. 28: 452-461.

Cralley, L.J., Key, M.M., Groth, D.H., Lainhart, W.S. and Ligo, R.M. 1968. Fibrous and mineral content of cosmetic talcum products. Am. Ind. Hyg. Assoc. J. 29: 350-354.

Dipple, A. 1976. Polynuclear aromatic carcinogens. In Chemical carcinogens. Edited by C.E. Searle. American Chemical Society, Washington, D.C. ACS Monograph 173. pp. 245-315.

Dixon, J.R., Lowe, D.B., Richards, D.E., Cralley, L.J. and Stokinger, H.E. 1970. The role of trace metals in chemical carcinogenesis: asbestos cancers. Cancer Res. 30: 1068.

Eneanya, D.I., Bernard, F.B. and Hart, R.W. 1979. Asbestos (chrysotile intermediate) alters the metabolism of benzo(a)pyrene in Syrian hamster embryo cells. Proceedings of the 11th International Congress of Biochemistry, Toronto. p. 687. Abstract 13-5-255. (Poster-13).

Epstein, S.S. 1974. Multiple factors in carcinogenesis. Environ. Health Perspect. 9: 331-333.

Furst, A. 1977. Inorganic agents as carcinogens. In Environmental cancer. Edited by H.F. Kraybill and M.A. Mehlman. Hemisphere Publishing Corporation, Washington. New York. pp. 209-230.

Grover, P.L., Hewer, A. and Sims, P. 1974. Metabolism of polycyclic hydrocarbons by rat-lung preparations. Biochem. Pharmacol. 23: 323-332.

Hammond, E.C., Selikoff, I.J., Lawther, P.L. and Seidman, H. 1976. Inhalation of benzo(a)pyrene and cancer in man. Ann. N.Y. Acad. Sci. 271: 116-124.

Harington, J.S. 1976. The biological effects of mineral fibers, especially asbestos, as seen from in vitro and in vivo studies. Ann. Anat. Pathol. 21: 155-198.

Harington, J.S., Allison, A.C. and Badami, D.V. 1975. Mineral fibers: chemical, physico-chemical and biological properties. Adv. Pharmacol. Chemother. 12: 291-409.

Harris, C.C. 1977. Respiratory carcinogenesis. In Lung cancer: clinical diagnosis and treatment. Edited by M.J. Straus. Grune and Stratton, New York. pp. 1-17.

Hart, R.W., Daniel, F.B., Kindig, O.R., Beach, C.A., Joseph, L.B. and Wells, R.C. 1980. Elemental modifications and polycyclic aromatic hydrocarbon metabolism in human fibroblasts. Environ. Health Perspect. 34: 59-68.

Hawco, F., Hulett, L. and O'Brien, P.J. 1980. Hydroxyl radical formation during P-450 function and benzo(a)pyrene hydroxylation. In Microsomes, drug oxidations and chemical carcinogenesis. Vol. I. Edited by M.J. Coon, A.H. Conney, R.W. Estabrook, H.V. Gelboin, J.R. Gillette and P.J. O'Brien. Academic Press, New York. 419-422.

Heidelberger, C. 1973. Current trends in chemical carcinogenesis. Fed. Proc. 32: 2154-2161.

Hill, D.L. and Shih, T.W. 1975. Inhibition of benzo(a)pyrene metabolism catalyzed by mouse and hamster lung microsomes. Cancer Res. 35: 2717-2723.

Holder, G., Yagi, H., Dansette, P., Jerina, D.M., Levin, W., Lu, A.Y.H. and Conney, A.H. 1974. Effects of inducers and epoxide hydrase on the metabolism of benzo(a)pyrene by liver microsomes and a reconstituted system: analysis by high pressure liquid chromatography. Proc. Natl. Acad. Sci. U.S.A. 71: 4356-4360.

Jaurand, M.C., Bignon, J., Sebastien, P. and Goni, J. 1977. Leaching of chrysotile asbestos in human lungs. Environ. Res. 14: 245-254.

Kandaswami, C. and O'Brien, P.J. 1980. Effects of asbestos on membrane transport and metabolism of benzo(a)pyrene. Biochem. Biophys. Res. Commun. 97: 794-801.

Kandaswami, C. and O'Brien, P.J. 1981. Pulmonary metabolism of benzo(a)pyrene: effect of asbestos. Biochem. Pharmacol. 30: 811-814.

Kouri, R.E., Salerno, R.A. and Whitmire, C.E. 1973. Relationship between aryl hydrocarbon hydroxylase inducibility and sensitivity to chemically induced subcutaneous sarcomas in various strains of mice. J. Natl. Cancer Inst. 50: 363-368.

Lakowicz, J.R. and Bevan, D.R. 1980. Effects of adsorption of benzo-(a)-pyrene to asbestos and non-fibrous mineral particulates upon its rate of uptake into phospholipid vesicles and rat liver microsomes. In The in vitro effects of mineral dusts. Edited by R.C. Brown, I.P. Gormley, M. Chamberlain and R. Davis. Academic Press, London. pp. 169-175.

Laskin, S., Kuschner, M. and Drew, R.T. 1970. Studies in pulmonary carcinogenesis. In Inhalation carcinogenesis. Edited by M.G. Hanna, P. Nettesheim and J.R. Gilbert. AEC Symposium Series 18, Oak Ridge, Tennesse, USAEC. pp. 321-352.

Morgan, A. and Cralley, L.J. 1974. Chemical characteristics of asbestos and associated trace elements. IARC Sci. Publ. 8: 113-118.

Naseem, S.M., Tishler, P.V., Anderson, H.A. and Selikoff, I.J. 1978. Aryl hydrocarbon hydroxylase in asbestos workers. Am. Rev. Respir. Dis. 118: 693-700.

Nebert, D.W. 1980. The Ah locus. A gene with possible importance in cancer predictability. In Quantitative aspects of risk assessment in chemical carcinogenesis. Arch. Toxicol. Suppl. 3. Springer-Verlag, Berlin. pp. 195-207.

Pylev, L.N., Roe, F.J.C. and Warwich, G.P. 1969. Elimination of radio-activity after intratracheal instillation of tritiated 3,4-benzo(a)pyrene in hamsters. Br. J. Cancer 23: 103-115.

Rahman, Q., Viswanthan, P.N. and Zaidi, S.H. 1977. Some new perspectives on the biological effects of asbestos. Environ. Res. 14: 487-498.

Reiss, B., Solomon, S., Weisberger, J.H. and Williams, G.M. 1980. Comparative toxicities of different forms of asbestos in a cell culture assay. Environ. Res. 22: 109-129.

Roy-Chowdhury, A.K., Mooney, T.F. and Reeves, A.L. 1973. Trace metals in asbestos carcinogenesis. Arch. Environ. Health. 26: 253-255.

Saffiotti, U. and Wagoner, J.K. 1976. Occupational carcinogenesis. Ann. N.Y. Acad. Sci. 271: 1-516.

Saffiotti, U., Cefis, F. and Kolb, L.H. 1968. A method for the experimental induction of bronchogenic carcinoma. Cancer Res. 28: 104-129.

Seidman, H., Selikoff, I.J. and Hammond, E.C. 1979. Short-term asbestos work exposure and long-term observation. In Health hazards of asbestos exposure. Edited by I.J. Selikoff and E.C. Hammond. The New York Academy of Sciences, New York. pp. 61-89.

Selikoff, I.J. and Lee, D.H.K. 1978. Asbestos and disease. Academic Press Inc., New York. pp. 307-336.

Selkirk, J.K., Croy, R.G., Roller, P.P. and Gelboin, H.V. 1974. High pressure liquid chromatographic analysis of benzo(a)pyrene metabolism and covalent binding and the mechanism of action of 7,8-benzoflavone and 1,2-epoxy-3,3,3-tri-chloropropane. Cancer Res. 34: 3474-3480.

Severson, R.F., Snook, M.E., Akin, F.J. and Chrotyk, O.T. 1978. Correlation of biological activity with polynuclear aromatic hydrocarbon content of tobacco smoke fractions. In Carcinogenesis: a comprehensive survey. Vol. 3: Polynuclear aromatic hydrocarbons. Edited by P.W. Jones and R.I. Freudenthal. Raven Press, New York. pp. 115-130.

Shabad, L.M., Pylev, L.N., Krivosheevo, T., Kulagina, F. and Nemenko, B.A. 1974. Experimental studies on asbestos carcinogenicity. J. Natl. Cancer Inst. 52: 1175-1180.

Shugar, S. 1979. Effects of asbestos in the Canadian environment. Associate Committee on Scientific Criteria for Environmental Quality. National Research Council of Canada. NRCC No. 16452. pp. 185.

Sims, P. 1970. Qualitative and quantitative studies on the metabolism of a series of aromatic hydrocarbons by rat-liver preparations. Biochem. Pharmacol. 19: 795-818.

Sims, P. and Grover, P.L. 1974. Epoxides in polycyclic aromatic hydrocarbon metabolism and carcinogenesis. Adv. Cancer Res. 20: 165-274.

Sincock, A.M. 1976. Preliminary studies of the in vitro cellular effects of asbestos and fine glass dusts. In Origins of human cancer. Edited by H.H. Hiatt, J.D. Watson and J.A. Winsten. Cold Spring Harbour Lab., Cold Spring Harbor, N.Y. pp. 289-294.

Sirois, P., Rola-Pleszczynski, M. and Bégin, R. 1980. Phospholipase A activity and prostaglandin release from alveolar macrophages exposed to asbestos. Prostaglandins Med. 5: 31-37.

Stanton, M.F. 1973. Some etiological considerations of fiber carcinogenesis. IARC Sci. Publ. 8: 289-294.

Sunderman, F.W. 1978. Carcinogenic effects of metals. Fed. Proc. 37: 40-46.

Tejwani, G.A., Fertel, R., Hart, R.W. and Allison, D.K. 1980. Effects of asbestos and chemical carcinogens on the cyclic nucleotide system of human fibroblasts. J. Environ. Pathol. Toxicol. 4: 67-70.

Thomson, R., Webster, I. and Kilroe-Smith, T.A. 1974. The metabolism of benzo(a)pyrene in rat liver microsomes: the effect of asbestos-associated metal ions and pH. Environ. Res. 7: 149-157.

Tishler, P.V., Naseem, S.M., Anderson, H.A. and Selikoff, I.J. 1977. Aryl hydrocarbon hydroxylase (AHH) in asbestos workers with high risk for lung cancer. Clin. Res. 25(3): A412.

Tsang, S. and Furst, A. 1976. In vitro inhibition of aryl hydrocarbon hydroxylase by heavy metals. Oncology 33: 201-204.

Weisburger, J.H. and Williams, G.M. 1980. Chemical carcinogens. In Toxicology: the basic science of poisons. Edited by J. Doull, C.D. Klaassen and M.O. Amdur. Macmillan Publishing Co., Inc., New York. pp. 84-138.

SOME EMERGING PRINCIPLES IN THE TOXICOLOGICAL ANALYSIS OF THE COMBINED EFFECTS OF XENOBIOTICS

E. Farber[a]

ABSTRACT

A brief analysis of some of the factors that determine the acute toxic response and the chronic carcinogenic response to xenobiotics is presented. The importance of xenobiotics as modifiers of essential steps in the multistep process of cell death and cancer development with xenobiotics is emphasized. Also, the problem of reconciling quantitative changes with qualitative toxic effects is presented. In the analysis of acute cell injury, emphasis is given to xenobiotics as modifiers by pretreatment and by post-treatment with each phase requiring a different orientation. In respect to chemical carcinogenesis, the influence of xenobiotic initiators as well as modifiers during different phases is considered. Some discrepancies between our current paradigm of chemical carcinogenesis and the observations with xenobiotic modifiers are briefly presented and discussed. It is emphasized in this presentation that processes, not only agents, must be studied and understood if a useful holistic view of the combined effects of xenobiotics is to be developed.

[a] Departments of Pathology and Biochemistry, University of Toronto, Toronto, Ontario M5G 1L5.

CERTAIN NOUVEAUX PRINCIPES POUR L'ANALYSE TOXICOLOGIQUES DES EFFETS COMBINES DES XENOBIOTIQUES

E. Farber[a]

RESUME

Brève analyse de certains des facteurs qui déterminent la réponse à la toxicité aiguë et la réponse à la carcinogénèse chronique des xénobiotiques. On insiste sur l'importance des xénobiotiques comme modificateurs d'étapes essentielles du processus à étapes multiples de la mort des cellules et sur le rôle des xénobiotiques dans le développement des cancers. On présente également le problème de la corrélation des changements quantitatifs et des effets toxiques qualitatifs. Dans l'analyse des lésions cellulaires aiguë, on s'attache aux xénobiotiques en tant que modificateurs par prétraitement et par post-traitement, chaque phase exigeant une orientation différente. En ce qui concerne la carcinogénèse chimique, on considère l'influence des initiateurs ainsi que des modificateurs xénobiotiques durant différentes phases. On fait brièvement état de quelques contradictions entre notre paradigme actuel de la carcinogénèse chimique et les observations avec des modificateurs xénobiotiques. La présentation fait ressortir qu'il convient d'étudier et de comprendre les processus, et non seulement les agents, si l'on veut avoir une vue holistique utile des effets combinés des xénobiotiques.

[a] Départements de pathologie et de biochimie, Université de Toronto, Toronto, Ontario M5G 1L5.

INTRODUCTION

Toxic manifestations of exposure to environmental agents are often the result of the mutual interaction between the agent and the target organism or tissue. Chemicals act on tissues, tissues act on chemicals and each in turn is subject to major modulation by diet, by other chemicals and by the physiological or pathological setting in which the interactions take place. This holistic orientation makes analysis as well as synthesis difficult. Nevertheless, it is essential if we are to understand and thus to suggest ways of minimizing toxicological consequences of possible hazards in our environment.

If this holistic orientiation is a valid one in the scientific analysis of toxicological phenomena, then at least three critical aspects come to mind: (a) the physico-chemical nature of the xenobiotic and its metabolism in the target tissues or organs; (b) the state of receptivity of the target tissues and its modulation, and (c) the qualitative and quantitative responses of the host tissues, especially those biological responses that relate to "toxicity". Each of these aspects needs to be studied if we are ever to understand the "combined effects of xenobiotics".

In this presentation, I shall concentrate on some aspects of (b) and (c) with little reference to (a). Since the focus will be on the scientific analysis of some of the major types of biological responses that constitute the toxicological consequences of some xenobiotics, little reference will be made to (a). This orientation seems relevant since the bulk of published work in this general area tends to concentrate on (a) (Coon et al. 1980) and not on (b) or (c) (Farber 1980a).

The number of xenobiotics is large. Many are lipid soluble, poorly metabolized and retained in the body for long periods of time. Others are readily metabolized and excreted. Some are highly toxic while others are much less so. Also, the bulk of research on xenobiotics to date has been on their metabolism and on their action as enzyme inducers. Are the important biological effects of exposure to xenobiotics, other than their acute toxicity, mediated largely or exclusively through enzyme induction? Since we know of few other possible mechanisms, this will constitute a major orientation in this presentation. Therefore, I shall confine my discussion largely to those xenobiotics that are enzyme inducers and shall restrict myself to levels of exposure that are not acutely toxic i.e. to low levels.

ENZYME INDUCTION

The properties of xenobiotics that have received most attention, other than their obvious acute or chronic pathological effects, are their ability to act as enzyme inducers. Despite the number

of studies in this area over the past 20 years, we remain remarkably ignorant about enzyme induction, even in one organ, such as the liver, and in one inducible system, the microsomal mixed function oxidase system (MFO) or the microsomal cytochrome P-450 system. Although investigators continue to refer to two major types of inducers, the phenobarbital (PB) type and the 3-methylcholanthrene (3-MC) types, this is a gross oversimplification. At least 4 different cytochrome P-450s are known and there is evidence that many more may exist (Lu 1979; Coon et al. 1980).

Also, a minimum of 8 polypeptides with at least 12 different patterns of induction can be identified when one looks at the protein profile of purified microsomes in the range of 45,000-60,000 daltons after the animals are exposed to a variety of xenobiotics including PB and 3-MC and other carcinogens (Sharma et al. 1979, 1981). At least 2 or 3 heme proteins and epoxide hydrolase have been identified. The analyses to date leave one with the uncomfortable feeling that we are far from a rational classification or categorization of the xenobiotic enzyme inducers using any set of parameters. This must not inhibit our deliberations and studies but should serve to introduce a healthy element of caution, uncertainty and scepticism.

We must also realize that the induction in the MFO system is only one of the possible consequences of the actions of xenobiotics. Other sites in the cells, such as peroxisomes and mitochondria, may also participate in the response patterns of cells. For example, there are an increasing number of drugs being used in the various types of hyperlipidemias. Included in this group are clofibrate, tibric acid, nafenopin and gemfibrozil. These compounds induce a large increase in liver peroxisomes with their contained enzymes, as well as some proliferation of the endoplasmic reticulum (see Reddy et al. 1980, de la Iglesia and Farber 1981). All of these compounds induce hepatocellular carcinoma and pancreatic neoplasms in rodents after long exposure. In view of the possible importance in some aspects of cell biochemistry and physiology of peroxisome enzymes such as catalase, carnitine acetyl transferase, enzymes involved in β-oxidation of long chain fatty acids and several hydrogen-peroxide generating oxidases, such as D-amino acid oxidase, it is not unreasonable to assume that xenobiotic peroxisome inducers may have important effects in modulating the toxicological consequences of exposure to some drugs and chemicals.

Very recently, the antihistaminic methapyrilene was reported to induce a proliferation of mitochondria in rodent liver, an organ that is also the site for carcinogenic effects of this drug (Reznik-Schüller and Sherman 1981).

Thus, our perspective in the study of xenobiotic as inducers and modifiers of toxic responses must be kept broad, even though we must focus on discrete phases if we are to understand phenomena from a mechanistic viewpoint. Major uncertainties concern the relation of these responses to exposure levels. Many of these responses, at the organelle

level as well as at the biochemical level of organization, are seen with fairly large doses. Are the responses qualitatively similar at the low levels of tissue concentration seen with the ubiquitous xenobiotics? Are the effects additive with combined exposure to such agents?

AGENTS AND PROCESSES

I also want to introduce early a concept I consider very important in the study of toxicological consequences of exposure to xenobiotics, the sequential stepwise analysis of the development of the biological end point under study, be it an acute reversible or irreversible lesion or a chronic process leading to cancer or other largely irreversible phenomena. Not only long-term processes such as carcinogenesis but also acute responses such as cell death are multistep in nature. It begins to appear that there is no direct relationship between the detailed nature of the initiating or triggering agent and the sequence of events that are triggered. There is what I call diversity of input and commonality of output or response. Living cells, tissues and organisms seem to have a limited number of ways in which they respond, even though the agents initiating the processes may belong to a very wide spectrum of chemical or physical agents.

This is important especially as we consider modulating effects of one xenobiotic on the toxicological responses to another xenobiotic. With stepwise processes, different steps can be modulated in different ways, even antagonistic ways. Thus, synergism or antagonism becomes predictable in the analysis of the combined biological effects of xenobiotics. A good illustration of this concerns the effects of PB on cancer development with chemicals. If carcinogenesis is initiated with 2-acetylaminofluorene, exposure to dietary PB (or DDT) accelerates or promotes cancer development (Peraino et al. 1971). However, if exposure to PB begins during initiation, cancer development is decreased or inhibited (Peraino et al. 1971). Similar apparent contradictions in effects have been reported with polychlorinated biphenyls (PCBs) (Makiura et al. 1974) and other xenobiotics (Wattenberg et al. 1976).

This concept immediately highlights the need to identify and assay for each important step in a process. The measurement of a final end point as an index of exposure to a xenobiotic in a multistep process may not only be insufficient but also misleading, at least from a mechanistic viewpoint.

Early in the modern era of studies on biochemical and molecular mechanisms of toxic responses, we all felt that a systematic "linear" approach would be a rational and logical one. By following what immediate changes an agent underwent and induced in tissues, we thought we could understand how a chemical might induce cell injury, cancer or other biological consequences. This approach has been very profitable in uncovering the metabolic activation of many types of xenobiotics. However, when the next steps are under study, it has become impossible to

select intelligently the options that are relevant to the toxic manifestation under study. We do not know how to relate the critical target for an activated form of an xenobiotic to the biological end point without the formulation of testable hypotheses. Clearly, both a forward as well as a backward approach, from the end point to a precursor based on hypothesis, are essential if we are ever to develop a rational way to examine the effects of xenobiotics.

In this context, it is important to stress that, given the number of xenobiotics that may be important, any approach not mechanistically based is not likely to help anyone, either the scientist or the regulator to arrive at a reasonable rational basis for control. It is predictable that the superficial cataloguing of phenomena using various combinations of xenobiotics at a range of dosages will not likely generate useful principles. For example, the further cataloguing of patterns of microsomal protein induction with various combinations of xenobiotics would seem to be without meaning unless one of these patterns can be interpreted in terms of their modulation of acute cellular or tissue toxicity or of chronic tissue responses such as cancer development.

QUANTITATIVE VERSUS QUALITATIVE CHANGES

One of the most difficult aspects in biology, including pathology and toxicology generally, is the relation between different quantitative levels of biochemical, physiological or structural changes induced by an agent and the cellular and tissue consequences in biological terms. This may be best illustrated by an example (Fig. 1). The exposure of a rat to a single dose of dimethylnitrosamine leads to methylation of DNA, RNA, protein and probably many other cell components and leads to death of liver cells. The degree of methylation is continuous and proportional to the dose. The appearance of cell death is discontinous, i.e. there is no detectable lethal response below a dose of 13 mg/kg but increasing cell death (necrosis) occurs with increasing dosage above this level (Farber *et al.* 1976). We cannot explain this phenomenon. Does the discontinuity in cell response reflect a similar discontinuity at the molecular level or is the relationship more subtle?

This phenomenon is obviously important in any analysis of multiple effects, since some pretreatment effects may shift the dose-response curve up or down and thus may have large biological consequences in response to a relatively minor biochemical change.

Such a close dependence of the type of response on dosage is not seen with every end point of cell injury with all xenobiotics. For example, with CCl_4, cell death, as one of three discrete hepatocellular effects of this xenobiotic, is seen with extremely small doses (Popp *et al.* 1978). However, with two other end points of cell injury induced by CCl_4, fatty change and balloon cells, each tissue response has its own dose-response curve (Popp *et al.* 1978). A similar phenomenon has

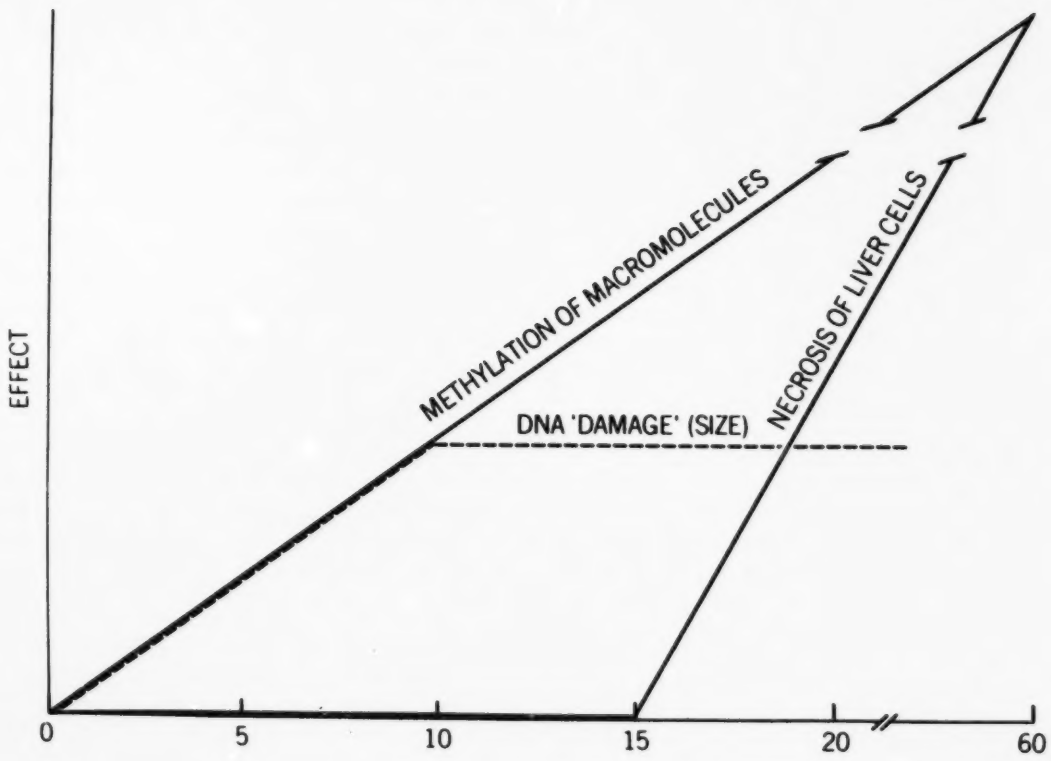


Figure 1. Schematic representation of the dose responses of 3 different effects of DMN in rat liver: methylation of a macromolecule, induction of DNA damage as measured by strand breaks in alkali (DNA size), and the induction of liver cell necrosis. Note especially the sharp onset of necrosis when the dose is increased above 15 mg/kg. Even a small decrease in the generation of the active moiety or moieties of DMA by the liver could result in a striking loss of a new qualitative effect, necrosis. (Reproduced with permission from Cancer Research, Farber et al. 1976). DMN, dimethylnitrosamine; abscissa: dose (mg/kg).

recently been observed in our laboratory. Post-treatment with diethyldithiocarbamate after treatment with a necrogenic dose of dimethylnitrosamine prevents cell death of hepatocytes in zone 3 of the liver but has no apparent protective effect upon fatty change in the hepatocytes in zone 1.

ACUTE TISSUE INJURY INCLUDING CELL DEATH

The induction of cell death under several circumstances appears to be a multistep process involving more than one cell organelle with the probable involvement of the plasma or cell membrane as an important late target (Farber 1971; Farber et al. 1971; Trump and Arstilla 1975; Popp et al. 1978; Farber 1979). The multistep nature appears to be valid both in "toxic" injury induced by xenobiotics and in ischemic necrosis (Chien and Farber 1977; Chien et al. 1977, 1978; Farber 1979).

Although the examples of the modulation of cell injury by xenobiotic agents are scarce, they highlight certain features worthy of emphasis. These examples will be divided in two groups: pretreatment and post-treatment, depending upon whether the xenobiotic modifier precedes or follows the administration of the xenobiotic that initiates the toxicologic response.

PRETREATMENT

Many chemicals require preliminary metabolic conversion to active derivatives (Slater 1978a) to initiate sequence leading to a toxic response. Thus, pretreatment with enzyme inducers might be expected to modify or enhance the toxic effects of other xenobiotics. The first clear-cut example of this was presented by McLean and McLean (1966) who showed that administration of PB or DDT would counteract the inhibition of hepatotoxicity of CCl_4 seen in rats on a protein-free diet. They subsequently reported the rapid induction of liver cirrhosis with PB plus CCl_4 ("instant cirrhosis") (McLean et al. 1969; McLean 1975). A similar enhancement of cytotoxicity, including cell death, has been reported for carbon disulphide (Bond and De Matteis 1969; Bond et al. 1969), halogenated aromatic hydrocarbons (Reid et al. 1973) including bromobenzene (Reid and Krishna 1973) and thioacetamide (Hunter et al. 1977). Also, several drugs such as halothane, acetaminophen (paracetamol) and furosemide can be made considerably more toxic by pretreatment with enzyme inducers (Gillette et al. 1974; Gillette 1980; Rao 1977).

In contrast, some inducers have the opposite effects and protect against some forms of acute injury. For example, 3-MC protects against the hepatotoxicity of bromobenzene (Zampoglione et al. 1973; Lau and Zannoni 1979) by modifying the balance between the genesis of toxic and nontoxic metabolites. Pregnenolone-16α-carbonitrite and 3-MC decrease the hepatotoxicity of CCl_4 (Schülte-Hermann 1974; Tuchweber et al. 1974).

The current view for the basis for these different effects of modifying xenobiotics on acute cell injury resides in their differing patterns of induction. In general, given the known complexity of one target system, the MFO system and the variety of patterns induced by different agents, such a diversity in the modulation of the toxic cell injury is not surprising. However, the underlying mechanism for each specific effect is unknown. Is the diversity in patterns of induction sufficient to account for the biological phenomena observed and if so, how?

An important determinant is the availability and concentration of trapping agents, such as glutathione (GSH), for the reactive metabolites generated. With some agents such as acetaminophen and carcinogens, the concentration of GSH can have a major influence in determining toxicity (Gillette 1979). In addition, pretreatment with small doses of an xenobiotic (e.g. CCl_4) can protect against a subsequent large dose, presumably by destroying the MFO system in the endoplasmic reticulum (Slater 1978b).

In addition, the state of receptivity of the target organelle or molecule can play an important influence. For example, CCl_4 has no major deleterious effect on the protein synthetic apparatus when the ribosomes are "frozen" on polysomes by agents such as cycloheximide or tenuazonic acid (Farber 1972). However, with normal movement of ribosomes to and from polysomes, the free ribosomes or the subunits are damaged by CCl_4. Presumably, the interaction between the activated form(s) of CCl_4 and the ribosomes or enzymes or cofactors active in protein-synthesis is facilitated when the ribosomal subunits are in their dissociated state.

POST-TREATMENT

This phase of acute injury is not well studied. However, it is evident that liver cells *in vitro* show no irreversible cell damage with several xenobiotics agents in the absence of Ca^{2+} (Schanne *et al.* 1979; Kane *et al.* 1980). Also, with alkylating carcinogens such as dimethyl- and diethylnitrosamine, necrosis can be almost completely prevented *in vivo* by post-treatment with diethyldithiocarbamate (DEDTC) (Ying *et al.* 1980). This chelating-trapping agent also inhibits metabolism of several xenobiotics. However, even when administered after the metabolism is virtually complete and the methylation or ethylation of DNA and other macromolecules is almost maximal, DEDTC is still effective in preventing cell death and the initiation of carcinogenesis (Ying *et al.* 1980, 1981). The necrosis triggers regeneration by cell proliferation, a phenomenon essential for initiation of liver carcinogenesis with chemicals (Farber 1980b). Some post-activation steps of these nitrosamines are being modulated so as to modify their toxicological consequences. There is no reason why similar phenomena may not be occurring with other initiating xenobiotics and modifiers.

CARCINOGENESIS

As with acute injury, cancer induction with chemicals can be modulated in major ways by pretreatment or post-treatment with xenobiotics.

Pretreatment or simultaneous treatment with PB, 3-MC, α-hexachlorocyclohexane, DDT or PCB are effective in preventing or delaying cancer development in the liver with several carcinogens such as 2-acetylaminofluorine (2-AAF), amino azo dyes, aflatoxin B_1 or bracken fern (Richardson et al. 1952; Miller et al. 1958; Pamucku et al. 1971; McLean and Marshall 1971; Peraino et al. 1971; Makuira et al. 1974; Thamavit et al. 1974) but enhances that induced by safrole (Wislocki et al. 1977).

In contrast, treatment with PB, DDT, or PCBs after initiation of liver carcinogenesis with 2-AAF, azo dyes or other carcinogens enhances cancer development by decreasing the time taken for cancer to appear and increasing cancer incidence (Peraino et al. 1971; Pitot and Sirica 1980). Carcinogens have the capability to both initiate and promote cancer development but non-carcinogens or weak carcinogens can substitute in the post-initiation phase. However, either PB or 3-MC is very effective in preventing the selective ability of dietary 2-AAF to stimulate initiated cells to develop into hyperplastic nodules in the promotion of liver cancer (Solt 1978; Cayama and Solt 1980).

Also, the discrepancy between the similarity in the effects of 3-MC and PB in the prevention of liver cancer and the dissimilarity in their effects on acute cell injury is noteworthy and puzzling. As already indicated, PB and 3-MC have opposite effects in modulating the acute toxic action of bromobenzene and CCl_4. In this same vein, PB, PCBs and other inducers increase the efficiency in the generation of mutagens from procarcinogens by suitable cell free preparations, yet prevent cancer initiation and development (Farber 1980a).

Clearly, PB, 3-MC or other xenobiotics can prevent cancer if given early, can prevent the promoting effect of a dietary carcinogen, 2-AAF if given with the carcinogen, or can promote or accelerate cancer development if given after initiation of carcinogenesis with one of several different carcinogens. What effects, if any, on carcinogenesis are being exerted by the low tissue levels of PCBs, DDT or other xenobiotics in the millions of humans in the western world? Are such xenobiotics at the continuing levels in the tissues influencing the receptivity or susceptibility of tissues to other xenobiotics during fetal life or at some subsequent stage in human development?

Thus, as of now, the effects of inducers on chemical carcinogenesis in the liver and on the genesis of acute injury are confusing and contradictory. Clearly, only a mechanistic approach at the enzymatic and molecular levels may enable a rational reconciliation of the data on carcinogenesis. That modifying xenobiotics can radically alter the

patterns of metabolism is clear. However, we have a strong suspicion that any single approach that concentrates on only one aspect such as the relative amounts of metabolites generated from an added xenobiotic will not in itself be interpretable, since the cellular consequence of an exposure to an xenobiotic may often be the resultant of the opposing action of several metabolic pathways.

Based on the experience to date in chemical carcinogens, a much more integrated approach using the collaboration of chemists and biologists is needed if this problem is to be approached in a manner that should generate knowledge, not merely isolated facts.

SOME EMERGING GENERAL PRINCIPLES

Certain needs and principles appear to be emerging for the scientific analysis of the toxic effects of xenobiotics, either singly or in combination, and for the use of such an analysis in its application in regulation. Some of these are as follows:

1. There is a need to develop and use reliable quantitative assays for toxic responses to two or more xenobiotics in various tissues or organs. These must include acute toxic end points and discrete early and late steps in the development of cancer or other chronic pathological consequences of exposure to xenobiotics.

2. There is an urgent need to relate biochemical changes induced by xenobiotics singly or in combination to discrete interpretable biological end points such as in principle 1. There is no evidence that the enzyme analyses or assays now being used as major indices in the study of xenobiotics are adequate to relate the biochemical effects to toxicty.

3. Toxic manifestations appear to be largely responses by cells and tissues, i.e. they require some integrated activity by cells. Thus, processes as well as agents must be studied if an adequate scientific base for understanding the combined effects of xenobiotics is to be developed.

4. Many if not all toxic responses to xenobiotics are multistep in nature. The steps should be studied in detail so that the modifying or modulating effects of xenobiotics on toxic responses to other xenobiotics can be scientifically analyzed. The "commonality of output" as related to the "diversity of input" in toxic effects of xenobiotics may thus be explored in detail.

5. Finally, biochemical and biological (cellular pathologic) studies should be pursued in the same species under the same experimental conditions in order to develop some basic relationships between different levels of organization in the genesis of acute cell injury and chronic disease such as cancer induced by xenobiotics.

SOME GENERAL CONSIDERATIONS

It is evident from this brief overview of a limited spectrum of problems that we must look to a different pattern of research if meaningful knowledge is to be generated. The close planning and cooperation between chemists, cellular physiologists and pathologists and others interested in this aspect of toxicology would seem to be essential.

Also, in my view, if we are ever to understand how to handle the combined toxicological effects of xenobiotics, our approach must be mechanistic, not descriptive. The number of variables are such that any approaches other than mechanistic ones can continue to generate pieces of information that will remain isolated pieces unless every effort is made to insert them into the proper place in the jigsaw puzzle. Unless the holistic viewpoint is realistically used, I do not see us making very much progress in this urgent problem that we all face, not only as scientists but also as people.

REFERENCES

Bond, E.J. and De Matteis, F. 1969. Biochemical changes in the rat liver after administration of carbon disulphide with particular reference to microsomal changes. Biochem. Pharmacol. 18: 2531-1549.

Bond, E.J., Butler, W.H., De Matteis, F. and Barnes, J.M. 1969. Effects of carbon disulphide on the liver of rats. Br. J. Ind. Med. 26: 335-337.

Cayama, E. and Solt, D.B. 1980. Promoting effect of 2-acetylaminofluorene plus liver mitogenic stimulus on heptacarcinogenesis induced by methylnitrosourea (MNU) and diethylnitrosamine (DENA). Proc. Am. Assoc. Cancer Res. 21: 73.

Chien, K.R. and Farber, J.L. 1977. Microsomal membrane dysfunction in ischemic liver cells. Arch. Biochem. Biophys. 180: 191-198.

Chien, K.R., Abrams, J., Pfau, R.G. and Farber, J.L. 1977. Prevention by chloropromozine of ischemic liver cell death. Am. J. Pathol. 88: 539-558.

Chien, K.R., Abrams, J., Serroni, A., Martin, J.T. and Farber, J.L. 1978. Accelerated phospholipid degradation and associated membrane dysfunction in irreversible ischemic cell injury. J. Biol. Chem. 253; 4809-4815.

Coon, M.J., Conney, A.H., Estabrook, R.W., Gelboin, H.V., Gillette, J.R. and O'Brien, P.J. (eds.) 1980. Microsomes, drug oxidations and chemical carcinogenesis. Vols. I and II. Academic Press, New York.

de la Iglesia, F.A. and Farber, E. 1981. Hepatocarcinogenesis of hypolipidemic agents. In Organ-directed toxicity. Chemical indices and mechanisms. Edited by S.S. Brown and D.S. Davies. Pergamon Press, Oxford. pp. 175-182.

Farber, E. 1971. Biochemical pathology. Ann. Rev. Pharmacol. 11: 71-96.

Farber, E. 1972. The pathology of translation. In The pathology of transcription and translation. Edited by E. Farber. Marcel Dekker, New York. pp. 123-158.

Farber, E. 1980a. Toxicological significance of liver hypertrophy produced by inducers of drug-metabolizing enzymes. In Environmental chemicals, enzyme functions and human disease. Ciba Foundation Symp. 76. pp. 261-274.

Farber. E. 1980b. The sequential analysis of liver cancer induction. Biochim. Biophys. Acta 605: 149-166.

Farber, E., Liang, H. and Shinozuka, H. 1971. Dissociation of the effects of protein synthesis and ribosomes from membrane changes induced by carbon tetrachloride. Am. J. Pathol. 64: 601-617.

Farber, E., Parker, S. and Gruenstein, M. 1976. The resistance of putative premalignant liver cell populations, hyperplastic nodules, to the acute cytotoxic effect of some hepatocarcinogens. Cancer Res. 36: 3879-3887.

Farber, J.L. 1979. Reactions of the liver to injury: necrosis. In Toxic liver injury. Edited by E. Farber and M.M. Fisher. Marcel Dekker, New York. pp. 215-241.

Gillette, J.R. 1979. Toxicological implications of drug metabolism. In The Induction of drug metabolism. Edited by R.W. Estabrook and E. Lindenlaub. Symp. Med. Hoechst 14, Schattauer, Stuttgart. pp. 309-325.

Gillette, J.R. 1980. An overview of the role of microsomal enzymes in the formation of toxic metabolites. In Microsomes, drug oxidations and chemical carcinogenesis. Vol. II. Edited by M.J. Coon, A.H. Conney, R.W. Estabrook, H.V. Gelboin, J.R. Gillette and P.J. O'Brien. Academic Press, New York. pp. 777-788.

Gillette, J.R., Mitchell, J.R. and Brodie, B.B. 1974. Biochemical mechanisms of drug toxicity. Ann. Rev. Pharmacol. 14: 271-288.

Hunter, A.C., Holscher, M.A. and Neal, R.A. 1977. Thiacetamide-induced hepatic necrosis: I. Involvement of the mixed-function oxidase enzyme system. J. Pharmacol. Exp. Ther. 200: 439-448.

Kane, A.B., Young, E.E., Schanne, F.A. and Farber, J.L. 1980. Calcium dependence of phalloidin-induced liver cell death. Proc. Natl. Acad. Sci. U.S.A. 77: 1177-1180.

Lau, S.S. and Zannoni, V.G. 1979. Hepatic microsomal epoxidation of bromobenzene to phenols and its toxicological implication. Toxicol. Appl. Pharmacol. 50: 309-318.

Lu, A.Y.H. 1979. Multiplicity of mammalian microsomal cytochromes P_{450}. Pharmacol. Rev. 31: 277-295.

Makuira, S., Aoe, H., Sugihara, S., Hirao, K., Arai, M. and Ito, N. 1974. Inhibitory effects of polychlorinated biphenyls on liver tumorigenesis in rats treated with 3'-methyl-4-dimethylaminoazobenzene, N-2-fluorenylacetamide and diethylnitrosamine. J. Natl. Cancer Inst. 53: 1253-1257.

McLean, A.E.M. 1975. The measurement of liver injury and protection with special reference to paracetamol, dimethylnitrosamine and carbon tetrachloride. In Pathogenesis and mechanisms of liver cell necrosis. Edited by D. Keppler. MTP, Lancaster. pp. 119-127.

McLean, A.E.M. and Marshall, A. 1971. Reduced carcinogenic effects of aflatoxin in rats given phenobarbitone. Br. J. Exp. Pathol. 52: 322-329.

McLean, A.E.M. and McLean, E.K. 1966. Effect of diet and 1,1,1,trichloro-2,2-bis-(p-chlorophenyl) ethane (DDT) on microsomal hydroxylating enzymes and on the sensitivity of rats to carbon tetrachoride poisoning. Biochem. J. 100: 564-571.

McLean, E.K., McLean, A.E.M. and Sutton, M.P. 1969. Instant cirrhosis: an improved method for producing cirrhosis of the liver in rats by simultaneous administration of carbon tetrachloride and phenobarbitone. Br. J. Exp. Pathol. 50: 502-506.

Miller, E.C., Miller, J.A., Brown, R.R. and MacDonald, J.C. 1958. On the protective action of certain polycyclic aromatic hydrocarbons against carcinogenesis by amino azo dyes and 2-acetylaminofluorene. Cancer Res. 18: 469-477.

Pamucku, A.M., Wattenberg, L.M., Price, J.M. and Bryan, G.T. 1971. Phenothiazine inhibition of intestinal and urinary bladder tumors induced in rats by bracken fern. J. Natl. Cancer Inst. 47: 155-160.

Peraino, C., Fry, R.J.M. and Staffeldt, E. 1971. Reduction and enhancement of phenobarbital of hepatocarcinogenesis induced in the rat by 2-acetylaminofluorene. Cancer Res. 31: 1506-1512.

Pitot, H.C. and Sirica, A.E. 1980. The stages of initiation and promotion in hepatocarcinogenesis. Biochim. Biophys. Acta 605: 191-215.

Popp, J.A., Shinozuka, H. and Farber, E. 1978. The protective effects of diethyldithiocarbamate and cycloheximide on the multiple hepatic lesions induced by carbon tetrachloride in the rat. Toxicol. Appl. Pharmacol. 45: 549-564.

Rao, G.S. 1977. A study of the mechanism of halothane-induced liver necrosis: role of covalent binding of halothane metabolites to liver proteins in the rat. J. Med. Chem. 20: 262-265.

Reddy, J.K., Azarnoff, D.L. and Hignite, C.E. 1980. Hypolipidaemic hepatic peroxisome proliferation form a novel class of chemical carcinogens. Nature 283: 397-398.

Reid, W.D. and Krishna, G. 1973. Centrolobular hepatic necrosis related to covalent binding of metabolites of halogenated aromatic hydrocarbons. Exp. Mol. Pathol. 18: 80-99.

Reid, W.D., Krishna, G., Gillette, J.R. and Brodie, B.B. 1973. Biochemical mechanism of hepatic necrosis by aromatic hydrocarbons. Pharmacology (Basel) 10: 193-214.

Reznick-Schüller, H.M. and Sherman, M. 1981. Methapyrilene-induced liver carcinogenesis in rats. Proc. Am. Assoc. Cancer. Res. 22: 74.

Richardson, H.L. Stier, A.R. and Borson-Nachtnebel, E. 1952. Liver tumor inhibition and adrenal histologic responses in rats to which 3'-methyl-4-dimethylaminoazobenzene and 20-methylcholanthrene was simultaneously administered. Cancer Res. 12: 356-361.

Schanne, F.A., Kane, A.B., Young, E.E., and Farber, J.L. 1979. Calcium dependence of toxic cell death: a final common pathway. Science 206: 700-702.

Schülte-Hermann, R. 1974. Induction of liver growth by xenobiotic compounds and other stimuli. CRC Crit. Rev. Toxicol. 3: 97-158.

Sharma, R.N., Cameron, R.G., Farber, E., Griffin, M.J., Joly, J.G. and Murray, R.K. 1979. Multiplicity of induction patterns of rat liver microsomal mono-oxygenases and other polypetides produced by administration of various xenobiotics. Biochem. J. 183: 317-327.

Sharma, R.N., Gurtoo, H.L., Farber, E., Murray, R.K. and Cameron, R.G. 1981. Effects of hepatocarcinogens and hepatocarcinogenesis on the activity of rat liver microsomal epoxide hydrolase with observations on the electrophoretic behaviour of this enzyme. Cancer Res. 41: 3311-3319.

Slater, T.F. 1978a. Biochemical studies on liver injury. In Biochemical mechanisms of liver injury. Edited by T.F. Slater. Academic Press, London. pp. 1-44.

Slater, T.F. 1978b. Mechanisms of protection. In Biochemical mechanisms of liver injury. Edited by T.F. Slater. Academic Press, London. pp. 745-801.

Solt, D.B. 1978. A biological analysis of chemical hepatocarcinogenesis. Ph.D. Thesis, Temple University.

Thamavit, W., Hiasa, Y., Ito, N. and Bhamarapravati, N. 1974. The inhibitory effects of α-benzene hexachloride on 3'-methyl-4-dimethylaminoazobenzene and DL-ethionine carcinogenesis in rats. Cancer Res. 34: 337-340.

Trump, B.F. and Arstilla, A.V. 1975. Cell membranes and disease processes. In Pathobiology of cell membranes. Vol. I. Edited by B.F. Trump and A.V. Arstilla. Academic Press, New York. pp. 1-103.

Tuchweber, B., Werriglaer, J. and Kourounakis, P. 1974. Effect of phenobarbital or pregnenolone-16α-carbonitrite (PCN) pretreatment on acute carbon tetrachloride hepatotoxicity in rats. Biochem. Pharmacol. 23: 513-518.

Wattenberg, L.W., Loub, W.D., Lam, L.K. and Spier, J.L. 1976. Dietary constituents altering the responses to chemical carcinogens. Fed. Proc. 35: 1327-1331.

Wislocki, P.G., Miller, E.C., Miller, J.A., McCoy, E.C. and Rosenkranz, H.S. 1977. Carcinogenic and mutagenic activities of safrole, 1'-hydroxysafrole and some known or possible metabolites. Cancer Res. 37: 1883-1891.

Ying, T.S., Sarma, D.S.R. and Farber, E. 1980. The sequential analysis of liver cell necrosis. Inhibition of diethylnitrosamine and dimethylnitrosamine induced acute liver cell death by post-treatment with diethyldithiocarbamate. Am. J. Pathol. 99: 159-174.

Ying, T.S., Sarma, D.S.R. and Farber, E. 1981. Role of acute hepatic necrosis in the induction of early steps in liver carcinogenesis by diethylnitrosamine. Cancer Res. 41: 2096-2102.

Zampaglione, N., Jollow, D.J., Mitchell, J.R., Stripp, B., Hamrick, M. and Gillette, J.R. 1973. Role of detoxifying enzymes in bromobenzene-induced liver necrosis. J. Pharmacol. Exp. Ther. 187: 218-227.

GLOSSARY

ACTH - corticotrophin
AHH - aryl hydrocarbon hydroxylase
BP - benzo(a)pyrene
DHT - dihydrotestosterone
ED50 (ED_{50}) - median effective dosage
EDTA - a metal chelating agent
FSH - follicle stimulating hormone
GH - growth hormone
GPT - glutamic-pyruvic transaminase
GSH - glutathione
ip - intraperitoneal
IPL - isolated perfused lung
LH - luteinizing hormone
LHRH - LH releasing hormone
MC(3-MC) - 3-methylcholanthrene
MED - minimally effective dosage
MFO - mixed function oxidase
necrosis - localized death of living tissue
NED - noneffective dosage
neoplasia - formation of tumors
OCT - ornithine carbamyl transferase
PB - phenobarbital
PCBs - polychlorinated biphenyls
PCDD - polychlorinated dibenzodioxin
PCDF - polychlorinated dibenzofuran
po - peroral
PRL - prolactin
SGPT - serum glutamic-pyruvic transaminase
TCDD - 2,3,7,8 tetrachlorodibenzo-p-dioxin
TSH - thyroid stimulating hormone

PUBLICATIONS

of the
du

Environmental Secretariat
Secrétariat de l'environnement

NRCC Associate Committee on Scientific Criteria for Environmental Quality
CNRC-Comité associé sur les critères scientifiques concernant l'état de l'environnement

NRCC No. / CNRC nº	
12678	Inventory of Pollution-Relevant Research in Canada. Interim Report, Vols. 1, 2. Etat de recherches sur la pollution au Canada. Rapport provisoire, Tomes 1, 2. 1972. 310 pp. $6.50.
14108	Inventory of Pollution-Relevant Research in Canada. Vols. 3, 4. Etat de recherches sur la pollution au Canada. Tomes 3, 4. 1975. 558 pp. $6.50.
12226	Environmental Fluoride (Special Report). 1971. 39 pp.
12226	Le fluor dans l'environnement. 1971. 41 pp.
13079	Detection and Inactivation of Enteroviruses in Water. 1973. 26 pp. $1.25.
13236	Détection et inactivation des entérovirus dans l'eau. 1973. 30 pp. $1.25.
13080	Problems and Selection of Topics for Compilation of Cause/Effect Data. 1973. 51 pp. $1.25.
13339	Problèmes et choix de thèmes en vue d'une compilation de données cause/effet. 1974. 45 pp. $1.25.
13501	The Effects of Pulp and Paper Wastes on Aquatic Life with Particular Attention to Fish and Bioassay Procedures for Assessment of Harmful Effects. 1973. 60 pp. $2.50.
13502	L'effet des déchets de pâtes et papier sur la vie aquatique. 1974. 75 pp. $2.50.
13566	A Criteria Digest on Radioactivity in the Environment. 1974. 53 pp. $1.50.
13567	Un résumé de critères sur la radioactivité dans l'environnement. 1974. 45 pp. $1.50.
13682	Lead in the Canadian Environment. 1973. 116 pp. $2.00.
13683	Le plomb dans l'environnement canadien. 1973. 130 pp. $2.00.
13684	Picloram: The Effects of Its Use as a Herbicide on Environmental Quality. 1974. 128 pp. $1.00.
13685	Piclorame: les effets de son utilisation comme herbicide sur l'état de l'environnement. 1974. 130 pp. $1.00.
13686	A Method of Monitoring Nationally for Possible Delayed Effects of Various Occupational Environments. 1974. 42 pp. $1.25.
13687	Une méthode de surveillance nationale des effets possibles, à long terme, du milieu professionnel sur la santé. 1974. 40 pp. $1.25.
13688	Environmental Cause/Effect Phenomena Relating to Technological Development in the Canadian Arctic. 1974. 125 pp. $2.00.
13689	Phénomènes environnementaux de cause à effet liés au progrès technique dans l'arctique. 1974. 128 pp. $2.00.
13690	The Application of the Phosphorus Loading Concept to Eutrophication Research. 1974. 42 pp. $1.25.
13691	Application du concept de la vitesse d'apport de phosphore par unité de surface à la recherche sur l'eutrophisation. 1974. 44 pp. $1.25.

Available through prepaid purchase orders from

En vente, moyennant commandes prepayées, au Service des

Publications, NRCC/CNRC
Ottawa, Ontario K1A 0R6

NRCC No.
CNRC nº

14094 Chlordane: Its Effects on Canadian Ecosystems and Its Chemistry. 1974. 189 pp. $1.00.

14095 Chlordane: ses effets sur les ecosystèmes canadiens et sa chimie. 1975. 192 pp. $1.00

14096 Photochemical Air Pollution: Formation, Transport and Effects. 1975. 224 pp. $2.50.

14097 La pollution photochimique de l'air: sa formation, son transport et ses effets. 1976. 203 pp. $2.50

14098 Endosulfan: Its Effects on Environmental Quality. 1975. 100 pp. $1.25.

14099 Endosulfan: ses effets sur l'état de l'environnement. 1976. 98 pp. $1.25.

14100 Waterborne Dissolved Oxygen Requirements and Criteria With Particular Emphasis on the Canadian Environment. 1975. 111 pp. $2.00.

14101 Exigences et critères relatifs à l'oxygène dissous dans l'eau, et leurs particularités à l'environnement canadien. 1976. 111 pp. $2.00.

14102 Methoxychlor: Its Effects on Environmental Quality. 1975. 164 pp. $2.00.

14103 Méthoxychlore: ses effets sur l'état de l'environnement. 1976. 161 pp. $2.00.

14104 Fenitrothion: The Effects of Its Use on Environmental Quality and Its Chemistry. 1975. 162 pp. $2.00.

14105 Fénitrothion: effets de son utilisation sur l'état de l'environnement, et sa chimie. 1976. 164 pp. $2.00.

14106 Arboviruses and Human Health in Canada. 1975. 35 pp. $1.25.

14107 Arbovirus pathogènes au Canada. 1976. 40 pp. $1.25.

14109 Waste Heat in the Aquatic Environment. 1975. 40 pp. $1.00.

14110 Pollution thermique de l'environnement aquatique. 1976. 68 pp. $1.00.

15015 Sulphur and Its Inorganic Derivatives in the Canadian Environment. 1977. 426 pp. $5.00.

15016 Le soufre et ses dérivés mineraux dans l'environnement canadien. 1978. 441 pp. $5.00.

15017 Effects of Chromium in the Canadian Environment. 1976. 168 pp. $2.00.

15018 Les effets du chrome dans l'environnement canadien. 1977. 162 pp. $2.00.

15019 The Effects of Alkali Halides in the Canadian Environment. 1977. 171 pp. $2.00.

15020 Les effets des halogénures alcalins dans l'environnement canadien. 1977. 171 pp. $2.00.

15021 The Effects of Petroleum Refinery Liquid Wastes on Aquatic Life, with Special Emphasis on the Canadian Environment. 1976. 77 pp. $2.00.

15022 Les effets des déchets liquides du raffinage du pétrole sur la vie aquatique, particulièrement dans l'environnement canadien. 1977. 86 pp. $2.00.

15023 NTA (Nitrilotriacetic Acid) - An Ecological Appraisal. 1976. 46 pp. $1.25.

15024 NTA (acide nitrilotriacétique) - une analyse écologique. 1977. 48 pp. $1.25.

15383 Effects of Noise on Man. 1976. 87 pp. $2.50.

15384 Les effets du bruit sur l'homme. 1977. 92 pp. $2.50.

15385 *Bacillus thuringiensis*: Its Effects on Environmental Quality. 1976. 133 pp. $2.00.

15386 *Bacillus thuringiensis*: ses effets sur l'état de l'environnement. 1977. 127 pp. $2.00.

15387 Medicare Data: Its Use in Defining the Effects of the Environment on Health. 1977. 164 pp. $2.50.

15388 Les statistiques de l'assurance-maladie et leur rôle dans la définition de l'influence de l'environnement sur la santé. 1978. 166 pp. $2.50.

NRCC No.
CNRC nº

15389 Fenitrothion: The Long Term Effects of Its Use in Forest Ecosystems - Current Status. 1977. 18 pp. $1.00

16074 Fénitrothion: effets à long terme de son utilisation dans les écosystèmes forestiers - situation actuelle. 1977. 20 pp. $1.00.

15391 Effects of Arsenic in the Canadian Environment. 1978. 363 pp. $2.00.

15392 Les effets de l'arsenic sur l'environnement canadien. 1979. 415 pp. $2.00.

16073 Proceedings of a Symposium on Fenitrothion: The Long-Term Effects of Its Use in Forest Ecosystems.
Comptes rendus du symposium sur le fénitrothion: les effets à long terme de son utilisation dans les écosystèmes forestiers.
1977. 628 pp. $10.00.

16075 Phenoxy Herbicides: Their Effects on Environmental Quality with Accompanying Scientific Criteria for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD). 1978. 440 pp. $2.00.

16076 Herbicides phénoxy - analyse de leurs effets sur l'état de l'environnement accompagnée de critères scientifiques à l'égard de la 2,3,7,8-tétrachlorodibenzo-p-dioxine (TCDD). 1979. 452 pp. $2.00.

16077 Polychlorinated Biphenyls: Biological Criteria for an Assessment of Their Effects on Environmental Quality. 1978. 172 pp. $2.00.

16078 Polychlorobiphényles: critères biologiques pour évaluer leurs effets sur l'état de l'environnement. 1979. 208 pp. $2.00.

16079 Ecotoxicology of Chlorpyrifos. 1978. 314 pp. $2.00.

16080 Ecotoxicologie du chlorpyrifos. 1979. 250 pp. $2.00.

16081 Environmental Fluoride 1977. 1978. 152 pp. $2.00.

16082 Le fluorure dans l'environnement, 1977. 1979. 175 pp. $2.00.

16446 Effects of Ultraviolet Radiation on Man. 1978. 90 pp. $2.00.

16447 Les effets du rayonnement ultraviolet sur l'homme. 1979. 91 pp. $2.00.

16448 The Biological Effects of Radio-frequency and Microwave Radiation. 1979. 244 pp. $5.00.

16449 Effets biologiques des radiofréquences et des micro-ondes. 1980. 244 pp. $5.00.

16450 The Aqueous Chlorination of Organic Compounds: Chemical Reactivity and Effects on Environmental Quality. 1978. 136 pp. $2.00.

16451 Chloration en milieu aqueux de composés organiques: réactivité chimique et effets sur l'état de l'environnement. 1979. 149 pp. $2.00.

16452 Effects of Asbestos in the Canadian Environment. 1979. 185 pp. $3.00.

16453 Les effets de l'amiante dans l'environnement au Canada. 1980. 202 pp. $3.00.

16454 Copper in the Aquatic Environment: Chemistry, Distribution, and Toxicology. 1979. 227 pp. $3.00.

16455 Le cuivre dans l'environnement aquatique: chimie, répartition et toxicologie. 1980. 249 pp. $3.00.

16736 Effects of Lead in the Environment - 1978. Quantitative Aspects. 1979. 779 pp. $5.00.

16737 Effets du plomb dans l'environnement - 1978. Aspects quantitatifs. 1980. 853 pp. $5.00

16738 Report of a Special Conference on Hearing Threshold Levels of "Isolated" Human Populations.
Comptes rendus du symposium spécial sur les niveaux d'audition des populations humaines "isolées".
1978. 104 pp. $3.00.

16739 Effects of Mercury in the Canadian Environment. 1979. 290 pp. $3.00.

16742 Effets du mercure dans l'environnement au Canada. 1980. 292 pp. $3.00.

NRCC No.
CNRC nº

16740 Carbofuran: Criteria for Interpreting the Effects of Its Use on Environmental Quality. 1979. 191 pp. $2.00.

16741 Carbofurane: critères pour interpréter les effets de son utilisation sur l'état de l'environnement. 1980. 184 pp. $2.00.

16743 Effects of Cadmium in the Canadian Environment. 1979. 148 pp. $3.00.

16744 Les effets du cadmium dans l'environnement canadien. 1979. 150 pp. $3.00.

16745 Effects of Lead in the Canadian Environment - 1978. Executive Report.
Effets du plomb dans l'environnement canadien - 1978. Rapport administratif.
1979. 24 pp. $1.00.

17581 Water Hardness, Human Health, and the Importance of Magnesium. 1979. 119 pp. $3.00.

17582 Dureté de l'eau, santé et importance du magnésium. 1980. 123 pp. $3.00.

17583 Phthalate Esters in the Aquatic Environment. 1980. 108 pp. $3.00.

17584 Esters phtaliques dans l'environnement aquatique. 1981. 110 pp. $3.00.

17585 Executive Reports: Effects of Chromium, Alkali Halides, Arsenic, Asbestos, Mercury, and Cadmium in the Canadian Environment. 80 pp.
Rapports Administratifs: effets du chrome, des halogénures alcalins, de l'arsenic, de l'amiante, du mercure, et du cadmium sur l'environnement canadien. 92 pp.
1980. $3.00.

17586 A Case Study of a Spill of Industrial Chemicals - Polychlorinated Biphenyls and Chlorinated Benzenes. 1980. $5.00.

17587 Effects of Yellow Phosphorus in the Canadian Environment. 1981. 110 pp. $3.00.

17588 Effets du phosphore blanc sur l'environnement canadien. 1981. 112 pp. $3.00.

17589 Zinc in the Aquatic Environment: Chemistry, Distribution, and Toxicology. 1981. 145 pp. $3.00.

17590 Le zinc dans l'environnement aquatique: chimie, répartition et toxicologie. 1981. 152 pp. $3.00.

18128 Intestinal Parasites of Man in the Canadian Environment. 1980. 69 pp. $2.00.

18129 Les parasites intestinaux de l'homme dans l'environnement canadien. 1980. 70 pp. $2.00.

18132 Effects of Vanadium in the Canadian Environment. 1980. 94 pp. $2.00.

18133 Effets du vanadium dans l'environnement canadien. 1981. 92 pp. $2.00.

18134 Radioactivity in the Canadian Environment. 1980. 129 pp. $3.00.

18135 La radioactivité dans l'environnement canadien. 1981. 133 pp. $3.00.

18156 The Report of the [illegible] Panel Convened by the National Research Council of Canada to Review the Spill of Polychlorinated Biphenyls at the Federal Pioneer Ltd. Site in Regina, Saskatchewan. 30 January 1980. 15 pp.

18467 Hydrogen Sulfide in the Atmospheric Environment: Scientific Criteria for Assessing its Effects on Environmental Quality. 1981. 196 pp. $5.00.

18469 Ethylenethiourea: Criteria for the Assessment of its Effects on Man. 1980. 149 pp. $3.00.

18470 Ethylène-thiourée: critères pour evaluer ses effets sur l'homme. 187 pp. $3.00.

NRCC No.
CNRC nº

18471 Pesticide-Pollinator Interactions. 1981. 190 pp. $5.00.

18472 Interactions entre pesticides et pollinisateurs. 1981. 223 pp. $5.00.

18475 Acidification in the Canadian Aquatic Environment: Scientific Criteria for Assessing the Effects of Acidic Deposition on Aquatic Ecosystems. 1981. 369 pp. $10.00.

18476 Acidification dans l'environnement aquatique au Canada: critères scientifiques pour évaluer les effets du dépôt acide sur les écosystèmes aquatiques. 1982. 377 pp. $10.00.

18568 Effects of Nickel in the Canadian Environment. 1981. 352 pp. $4.00.

18569 Effets du nickel sur l'environnement canadien. 1982. 347 pp. $4.00.

18570 A Screen for the Relative Persistence of Lipophilic Organic Chemicals in Aquatic Ecosystems - An Analysis of the Role of a Simple Computer Model in Screening.
I. A Simple Computer Model as a Screen for Persistence.
II. An Introduction to Process Analyses and Their Use in Preliminary Screens of Chemical Persistence. 1981. 302 pp. $8.00.

18572 Effects of Propoxur on Environmental Quality with Particular Reference to its Use for Control of Biting Flies. 1982. 238 pp. $8.00.

18574 Polychlorinated Dibenzo-*p*-Dioxins: Criteria for Their Effects on Man and his Environment. 1981. 251 pp. $8.00.

18576 Polychlorinated Dibenzo-*p*-Dioxins: Limitations to the Current Analytical Techniques. 1981. 184 pp. $8.00.

18578 Chlorinated Phenols: Criteria for Environmental Quality. 1982. 191 pp. $8.00.

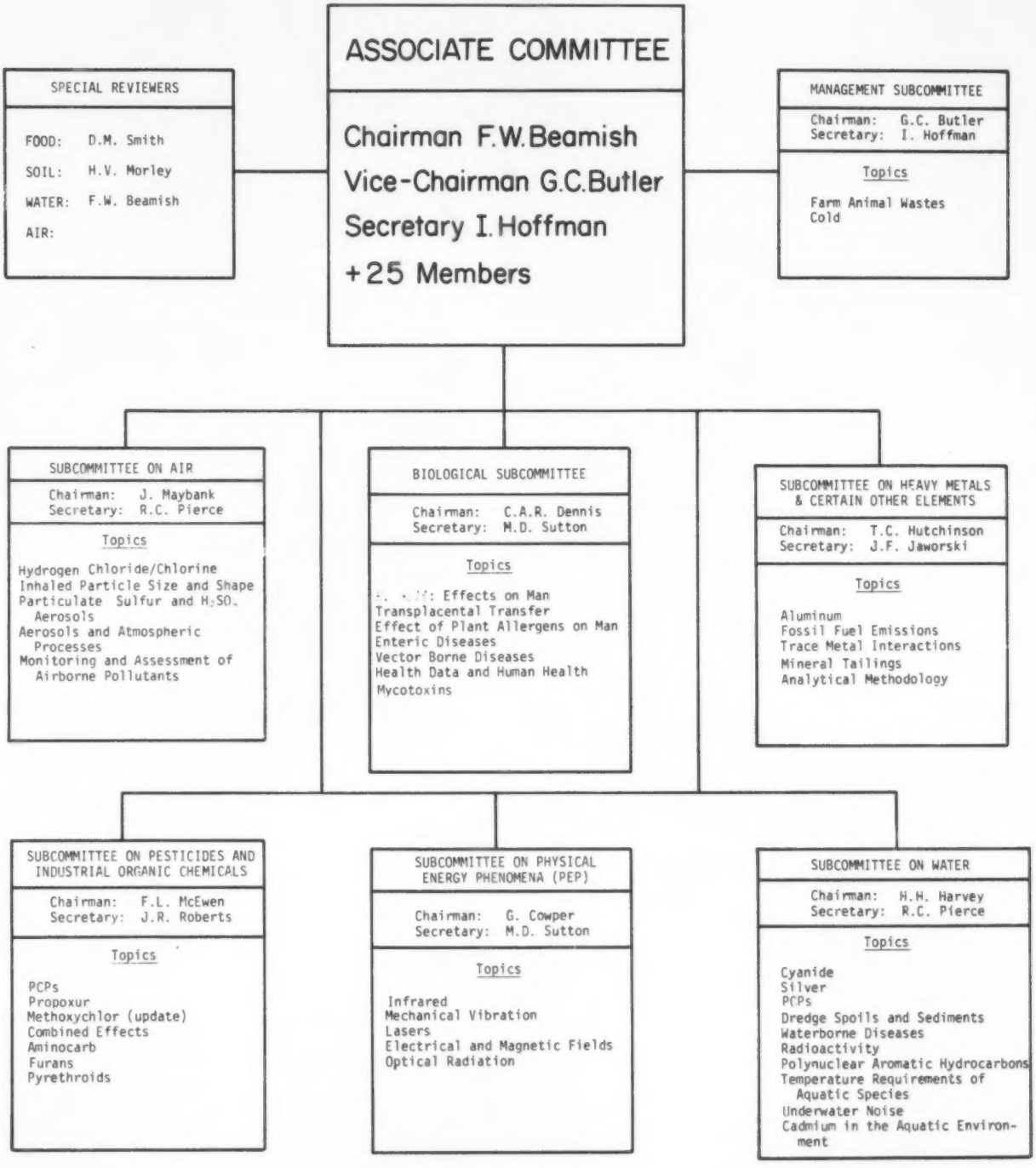
19244 Ultrasound: Characteristics and Biological Action. 1981. 284 pp. $8.00.

19245 Les ultrasons: caractéristiques et action biologique. 1982. 284 pp. $8.00.

Available through prepaid purchase orders from

En vente, moyennant commandes prepayées, au Service des

Publications, NRCC/CNRC
Ottawa, Ontario K1A 0R6

ORGANIZATION CHART

ASSOCIATE COMMITTEE ON SCIENTIFIC CRITERIA FOR ENVIRONMENTAL QUALITY